September 1956 · VOL. 15 · No. 3

Federation Proceedings

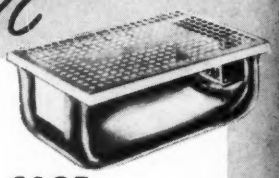
CONTENTS

Notes on the Fortieth Annual Meeting	875
Problems of Scientific Manpower	877
National Register of Scientific and Technical Personnel	880
Federation Placement Service	881
Joint Session of the Federation	885
Inter-Society Session on Nutritional and Toxicological Studies on Irradiated Foods	905
Symposium on Biology of Aging	938
Symposium on Amino Acids and Proteins	965
New Members Elected, Spring 1956	986
Corrections and Additions to March Issue	991

Published quarterly by the
FEDERATION OF AMERICAN SOCIETIES
for EXPERIMENTAL BIOLOGY

XUM

Leading Laboratories *Prefer* MOUSE-HOUSE



THE *Plastic* ANIMAL CAGE

because..

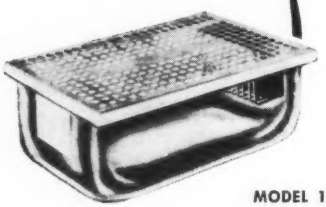
1. **SPEEDS RESEARCH**—clear, plastic walls permit daily cage inspection, aid observation of rodent development. False mesh bottoms speed metabolism tests and keep animals clean.

2. **CLEANER** — lower portion of cage is free from crevices and corners, thus cleaning usually involves only (1) turning cage upside down and (2) brushing unclean surfaces with hot soap suds and rinsing in hot water. Body acids and urine do not affect plastic cages. We recommend our HYTRON as a disinfectant.

3. **SAFER** — slow changing temperatures of smooth plastic walls protect rodents against drafts, and sudden temperature changes, thus reducing mortality rate.

4. **VERMIN PROOF** — the plastic MOUSE-HOUSE automatically eliminates the outside enemies to highly technical research.

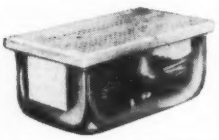
5. **LESS COSTLY** — by comparison with utility value of other cages. With reasonable handling these cages last indefinitely, making them more economical.



MODEL 150
STANDARD

CLEAR PLASTIC CAGE FOR RATS AND HAMSTERS. Molded from crystal clear plastic sheets; interchangeable stainless steel perforated cover fits snugly on the fluted sides of cage and has two special openings for inserting drinking tubes. Stainless steel feed cup fits over the end of the cage, inside the top rim, has a slotted base and a suspended hood. Permanent record card on end. Cage size for all 150 Models 11½" x 18½" x 6½".

Follow the lead of a large majority of research departments in leading universities, hospitals, privately endowed institutions and government bureaus who are regular users of this modern plastic animal cage. Everyone who uses this practical economical way of caging experimental rodents say the MOUSE-HOUSE cage is superior in every way.

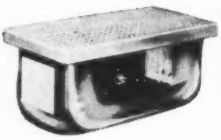


MODEL 101

CLEAR PLASTIC STOCK CAGE FOR MICE. Has all the construction features described for Model 150, but is smaller. Accommodates small rodents, cage size 11½" x 7½" x 5".

CLEAR PLASTIC CAGE FOR RATS AND HAMSTERS. Has same features of other plastic cages plus a stainless perforated slide-on cover with built-in food hopper which can be filled without opening the cage, plus a removable expanded metal grate above the plastic bottom. Grate is removable for cleaning. Size 13" x 9½" x 7".

MODEL 110

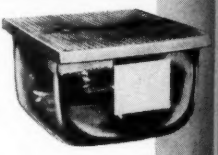


MODEL 103 STANDARD

CLEAR PLASTIC CAGE FOR MICE. This cage has all the construction features described for Model 101 except the size is smaller. Cage size for all 103 Models 11⅞" x 5⅛" x 5".

MODEL 102 STANDARD

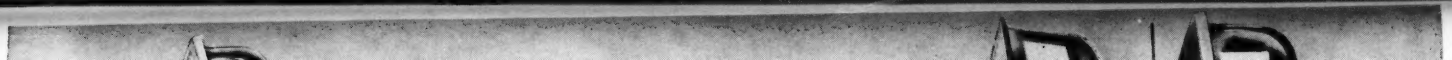
CLEAR PLASTIC CAGE FOR MICE. Has all the construction features described for Model 101 except the general shape is square instead of rectangular. Cage size for all 102 Models 11¼" x 11¼" x 6½".

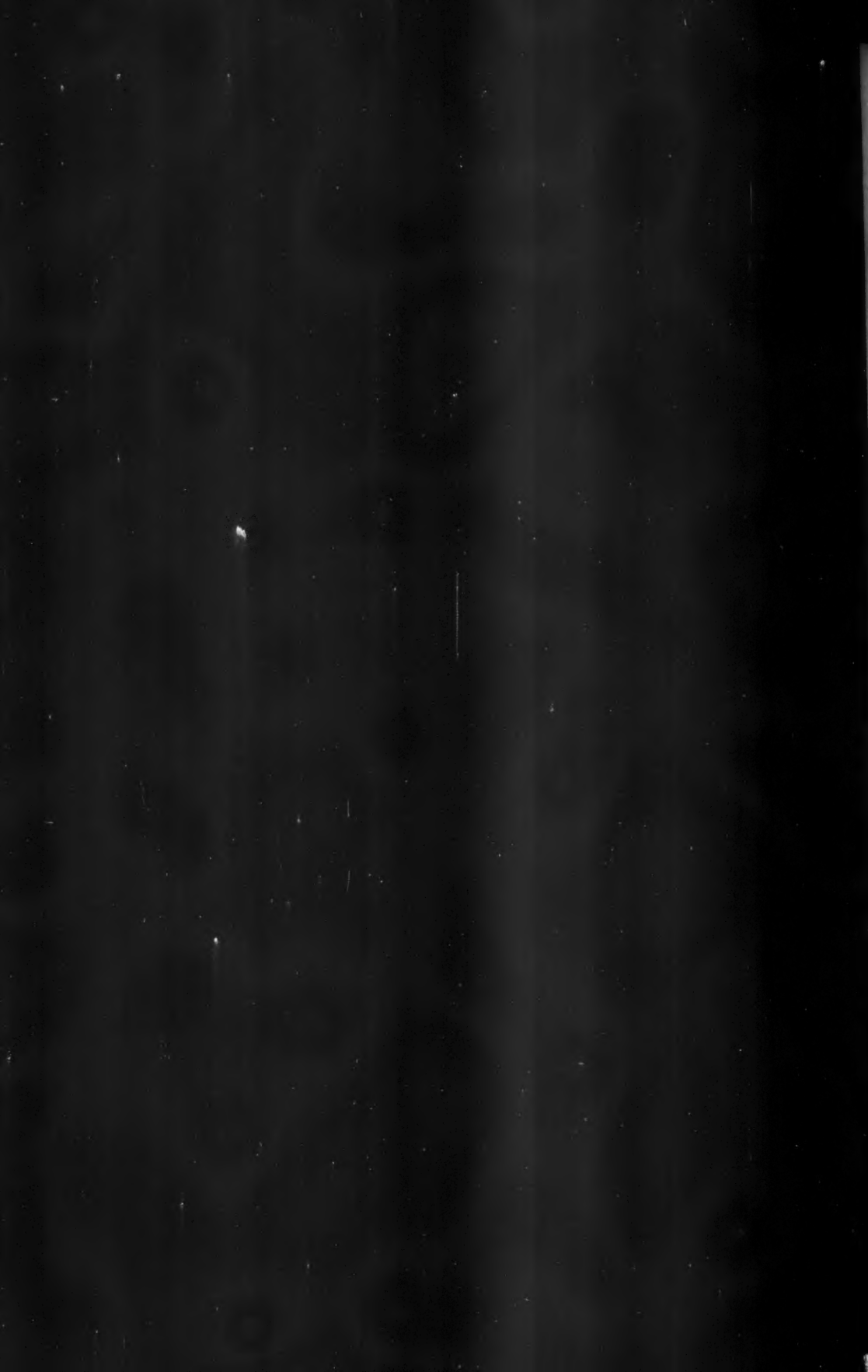


In addition to the Standard Models shown all MOUSE-HOUSE cages are available with a variety of metal covers especially designed for the varying needs of animal care and observation. Write today for more information.

Keystone Plastics Co. MEDIA, PA.

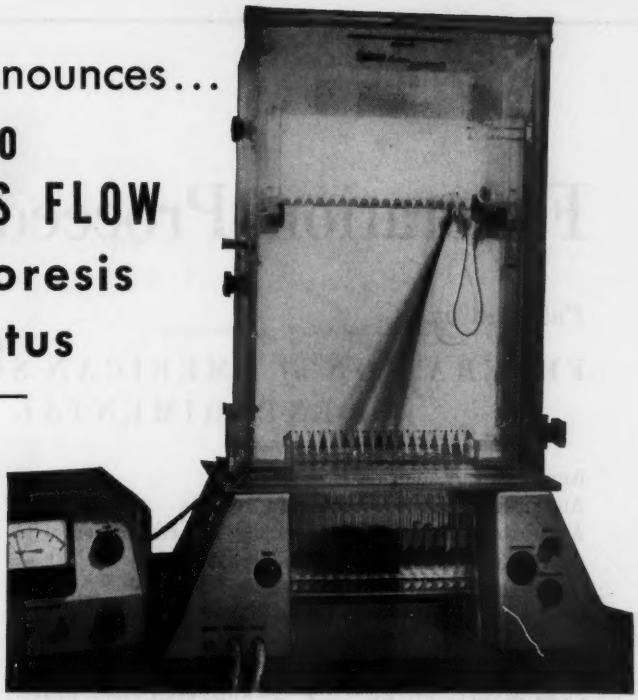
Specialists in Scientific Plastic Processing





Thomas announces...

Spinco CONTINUOUS FLOW Electrophoresis Apparatus



- **High resolution, flexibility and efficiency**
- **Precisely controlled sample feed and electrolyte flow**

CONTINUOUS FLOW PAPER ELECTROPHORESIS APPARATUS, Spinco Model CP (Patented). A new design which offers improvements in flexibility and efficiency. New methods of electrolyte feed and wide-range controls permit the instrument to give maximum throughput under any required degree of resolution. Design efficiency permits resolutions formerly requiring as much as 1500-volt operation to be made at one-third this value.

Controls permit precise adjustment of feed point and positioning and orientation of the pattern. Large-volume vertical wicks between the curtain and electrode vessels provide optimal distribution of the electrical field and prevent migration into the paper curtain of electrode products.

Applicable to the separation of all kinds of charged substances such as proteins, polypeptides, amino acids and other biological materials. Also suitable for organic substances such as dyes and intermediates. Particularly useful for following organic reactions and for immuno-chemical work.

Paper curtain electrophoresis offers the basic advantage that the sample travels a unique path and thus adsorption does not interfere with the purity of the product. Advantages of this new design are:

Easily assembled and disassembled for cleaning.

Stability of operation permits unattended runs of indefinite duration.

Paper curtains are quickly removable for oven drying.

Large samples can be processed at throughputs up to 8 ml per hour, small samples with virtually no hold-up.

400-000. Continuous Flow Paper Electrophoresis Cell, Spinco Model CP, complete with platinum wire electrodes but without power supply **1,220.00**

400-500. Power Supply, Spinco, for 100 to 1000-volt operation of above Cell; for 115 volts, 50/60 cycles, a. c. **490.00**

301-100. Duostat Power Supply, for 0 to 500-volt operation of above Cell; for 115 volts, 50/60 cycles, a. c. **200.00**

More detailed information sent upon request.

QUALITY AND SERVICE
A.H.T.CO.
PHILA. U.S.A.
LABORATORY APPARATUS

More and More Laboratories **RELY ON THOMAS**

ARTHUR H. THOMAS COMPANY

Laboratory Apparatus and Reagents

P. O. BOX 779 **PHILADELPHIA 5, PA.**

Teletype Services: Western Union WUX and Bell System TWX PH-72

XUM

Federation Proceedings

Published by

FEDERATION *of* AMERICAN SOCIETIES
for EXPERIMENTAL BIOLOGY

American Physiological Society
American Society of Biological Chemists
American Society for Pharmacology and Experimental Therapeutics
American Society for Experimental Pathology
American Institute of Nutrition
American Association of Immunologists

EDITORIAL BOARD

F. S. CHEEVER
R. W. ENGEL
C. C. ERICKSON
PHILIP HANDLER
HAROLD C. HODGE
LOUIS N. KATZ
MILTON O. LEE
Managing Editor

FEDERATION PROCEEDINGS is published quarterly. The *March* issue consists of the Abstracts (Part I) of the papers presented at the scientific sessions, and the Program (Part II) of the Annual Meeting of the Federation. The *July* and *September* issues contain symposia and other special papers presented at Federation meetings as selected by the Editorial Board. The *December* issue contains the membership list and other matters pertinent to the Constituent Societies of the Federation.

Entered as second class matter March 16, 1942, at the Post Office at Washington, D. C. under the Act of March 3, 1879. Copyright 1956, by the Federation of American Societies for Experimental Biology. Printed at Waverly Press, Baltimore, Md. All rights reserved.

SUBSCRIPTION PRICE: $7.50 per year ($8.00 Canada, $8.50 foreign) payable in advance. **Single Issues:** *March*, Part I (Abstracts) $5.00, Part II (Program) $1.00; *July* and *September*, $2.00 each; *December*, $3.00. Subscriptions and orders should be sent to:

Federation Proceedings

9650 Wisconsin Avenue **Washington 14, D. C.**

from

POETRY...

"...in today already walks tomorrow."

COLERIDGE

to

PHILOSOPHY...

"...most human activities advance by virtue of contributions from many different types of individuals, with vastly different endowments, working at different levels. Medical investigation is no exception to this rule."*

to

PHARMACEUTICALS

"In the research activities of the pharmaceutical manufacturers, therapeutic trends based on fundamental advances in biology and medicine clearly modify continually the subjects and directions of research."**

H. L. Daiell, M.D.
Scientific Director

P. S. Lakeside Laboratories specializes in the synthesis of new and distinctive compounds designed for "tomorrow's" therapy of cardiorenal, gastrointestinal, ventilatory and other disorders.

*The American Foundation: Medical Research: A Midcentury Survey, Boston, Little, Brown and Company, 1955, vol. 1, p. XXXI.

**Ibid., p. 600.

98456

We are pleased to announce a

New Colorimetric Procedure

for

SERUM TRANSAMINASE

—operates at 505 mμ

—does not require an ultraviolet spectrophotometer

— simple . . . rapid . . . accurate . . . inexpensive

Write for our NEW Bulletin No. 505.

Call Sigma for reagents for any Transaminase Procedures.

NOW AVAILABLE—CRYSTALLINE URIDINE DIPHOSPHATE—CHOLINE

—of Interest in the Biosynthesis of Phospholipids.

BE SURE TO GET YOUR FALL 1956 CATALOG LISTING PREVIOUSLY UNAVAILABLE COMPOUNDS

PHONE COLLECT: DAN BROIDA, ST. LOUIS, MISSOURI

Office: PRospect 1-5750 **Home: WYdown 3-6418**

SIGMA

CHEMICAL COMPANY

3500 DeKALB ST., ST. LOUIS 18, MO., U. S. A.

MANUFACTURERS OF THE FINEST BIOCHEMICALS AVAILABLE

Just Published

ANNUAL REVIEW OF

BIOCHEMISTRY

JULY, 1956

Vol. 25, 794 pages

Editors: J. M. LUCK, F. W. ALLEN, G. MACKINNEY

CONTENTS:

Prefatory Chapter—Sir Edward Mellanby, *B. S. Platt*
Nonoxidative and Nonproteolytic Enzymes, *A. Meister*
Proteolytic Enzymes, *J. S. Fruton and M. J. Mycek*
Carbohydrate Chemistry, *E. J. Bourne and R. Stephens*
Chemistry of the Lipides, *F. B. Shorland*
Metabolism of Purines and Pyrimidines, *C. E. Carter*
Biochemistry of Viruses, *F. W. Putnam*
Metabolism of Lipides, *S. Bergström and B. Borgström*
Biochemistry of Cellular Particles, *W. C. Schneider and G. Hogeboom*
Chemistry of the Fungi, *H. Raistrick and C. E. Stickings*
Biological Oxidations, *S. F. Velick*
The Chemistry of Proteins and Peptides, *H. Fraenkel-Conrat*
The Hemoglobins, *H. A. Itano*
Metabolism of Amino Acids and Proteins, *E. A. Adelberg and M. Rabinovitz*
Water-Soluble Vitamins, Part I, *J. J. Pfiffner and O. D. Bird*
Water-Soluble Vitamins, Part II, *E. E. Snell and D. E. Metzler*
Water-Soluble Vitamins, Part III, *G. W. E. Plaut and J. J. Betheil*
Fat-Soluble Vitamins, *E. Kodicek*
Nutrition, *W. H. Griffith and M. E. Swendseid*
The Biochemistry of Cancer, *C. Heidelberger*
Cholesterol Metabolism, *M. Friedman, S. O. Byers, and S. St. George*
Chemical Constitution and Immunological Specificity, *M. Heidelberger*
Metabolism of Drugs and Other Organic Substances, *W. H. Fishman*
Carbohydrate Metabolism, *S. Korkes*

PLANT PHYSIOLOGY

JUNE, 1956

Vol. 7, 456 pages

Editors: L. R. BLINKS, L. MACHLIS, J. G. TORREY

CONTENTS:

Mineral Nutrition of Plants: Mechanisms of Uptake and Transport, *E. Epstein*
Energy Transformations in Photosynthesis, *L. N. M. Duysens*
Nitrogen Fixation by Photosynthetic Organisms, *G. E. Fogg*
Endogenous Rhythms in Plants, *E. Bünning*
The Physiology of Cell Division, *H. Stern*
Obligate Parasitism, *C. E. Yarwood*
Biological Relations of Plant Viruses, *C. W. Bennett*
Iron Chlorosis, *J. C. Brown*
Photoperiodism in Woody Plants, *P. F. Wareing*
Plant Proteins, *C. E. Danielson*
Physiology of Root Elongation, *J. G. Torrey*
Mechanisms of Carbohydrate Breakdown in Plants, *B. Axelrod, and H. Beevers*
Physiology of Seed Germination, *E. H. Toole, S. B. Hendricks, H. A. Borthwick and V. K. Toole*
Phosphorus Metabolism and Photosynthesis, *D. I. Arnon*
Absorption and Translocation of Plant Regulators, *J. van Overbeek*
Effects of Light Quality on Plant Growth, *E. C. Wassink and J. A. J. Stolwijk*
Recent Advances in Plant Physiology in the U.S.S.R., *A. L. Kursanov*

Most Back Issues Available

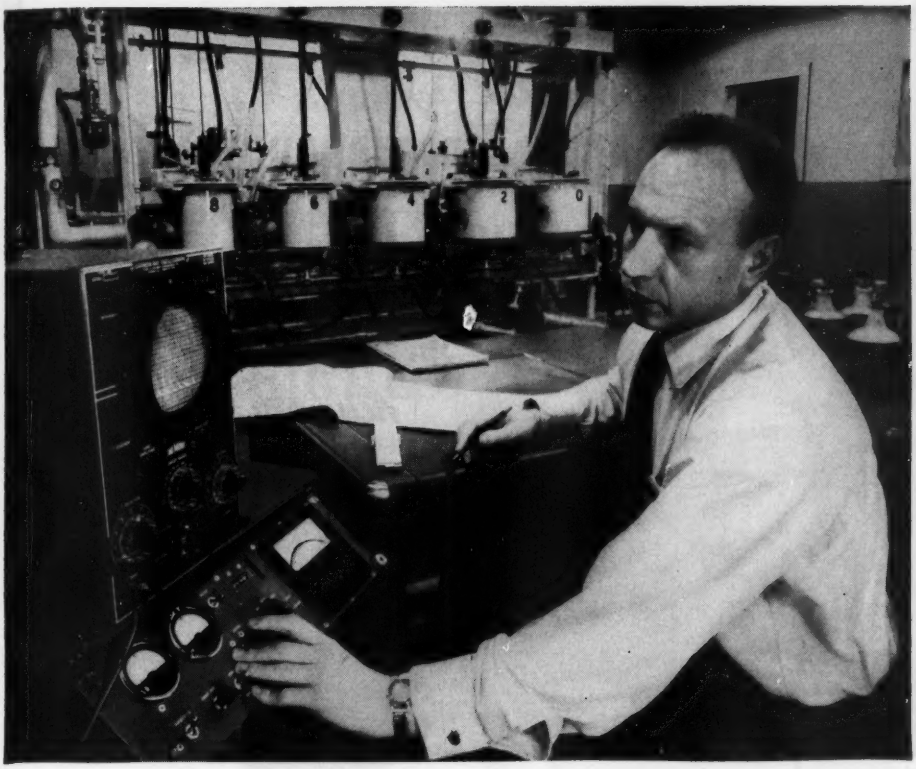
$7.00 postpaid (U.S.A.) **$7.50 postpaid (elsewhere)**

ANNUAL REVIEWS, INC.

GRANT AVENUE, PALO ALTO, CALIFORNIA

Pure as sunlight

Have a Coke...



Automation in pharmaceutical research

Automation is now playing an increasingly important role in pharmaceutical research. At Upjohn, a new electronic analog computer has been developed with the hope of facilitating hormone synthesis and the isolation of new antibiotics.

The need for an entirely new type of computer became evident when Upjohn research personnel developed a theoretical method of operating a 20-foot fractionating column which, if proved practical, would make possible the separation of highly complex mixtures. However, some quicker way of checking than either trial-and-error or repetitive arithmetic was needed to evaluate this theoretical operation against orthodox methods of operating the column. The solution was offered by Upjohn physicists, who worked out a relatively simple, inexpensive circuit for a computer that would calculate the problems, not in the adding-subtracting sense, but by forcing electrical energy to behave according to established laws of liquid extraction.

The electronic simulator does its work three to four hundred times as fast as a human with pencil and slide rule; expediency that may bring about an earlier appearance of certain types of new and improved pharmaceuticals.

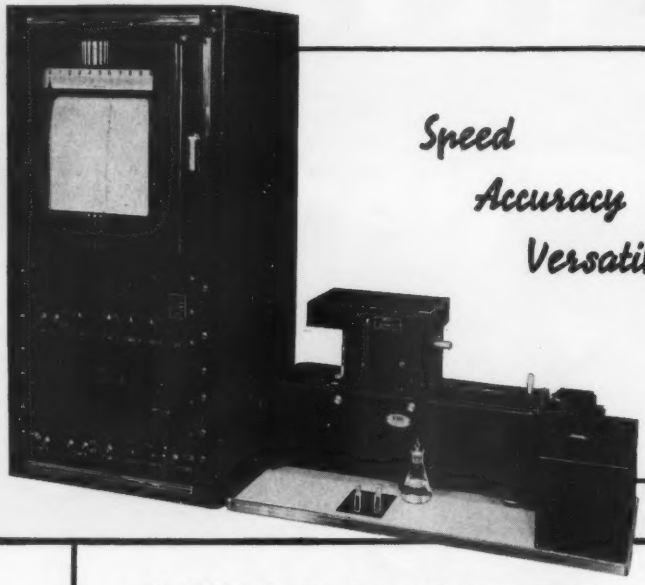
Upjohn

medicine . . .
designed for health . . .
produced with care

P&I *Automatic*

RECORDING SPECTROPHOTOMETER

Model RS-3*



Speed

Accuracy

Versatility

The P&I Recording Spectrophotometer RS-3 converts your present manual Beckman DU to a more sensitive line operated automatic recording instrument at a low cost.

High accuracy—stability—resolution simplify and accelerate compound identification and permit quantitative studies of analytical procedures, rapid kinetics, extinction coefficients, and differential colorimetry.

Only the P&I RS-3 provides **ALL** these unique features:

- **Linear Wavelength Scale—** no special charts or tapes.
- **Stable Zero Line**
- **Both O.D. & % Abs. are linear** 4 stand. & high sens. scales
- **Constant resolution**
- **Uninterrupted scan, 210-750 mμ.** no moving light sources
- **Automatic marking— every 20 mμ.**
- **Low-Med-High Scan Speeds**
- **Time Scale for Kinetics**
- **Line Operated**

For details, write:

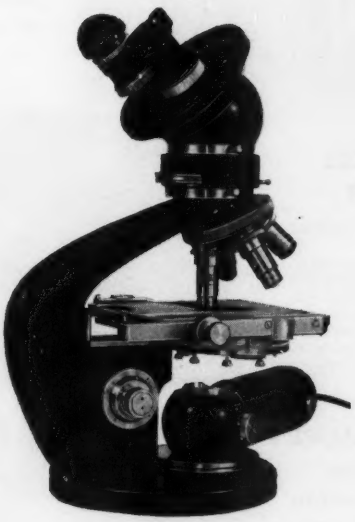
PROCESS & INSTRUMENTS

15 Stone Ave., Brooklyn 33, N. Y.

*Patent Pend.

MADE IN CARL ZEISS WEST GERMANY

LABORATORY and RESEARCH MICROSCOPE OF HIGHEST PRECISION



MODEL W

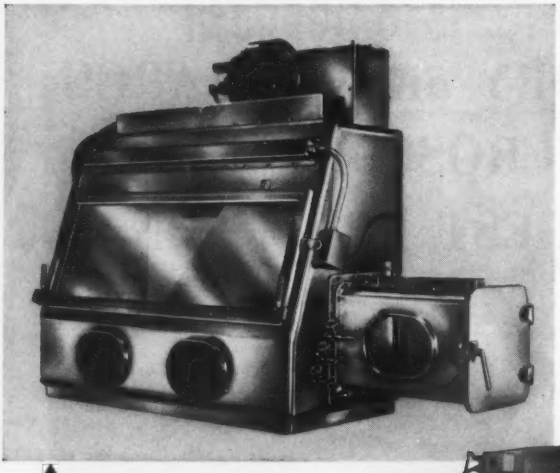
Truly the most COMPLETE and VERSATILE microscope of its kind. In fact, there is so much to say about this magnificent instrument, that only a booklet can begin to list its many features and advantages.
You are invited to write for your free copy immediately . . . and learn why we take justifiable pride in presenting the CARL ZEISS LABORATORY and RESEARCH MICROSCOPE MODEL W.

CARL ZEISS, INC. 485 5th Ave., New York 17, N.Y.

Guaranteed uninterrupted repair service

NEW SAFEGUARDS FOR THE LABORATORY

Stainless steel enclosures for handling hazardous substances



SAFETY is the prime factor in this newly-designed special-purpose equipment. These two enclosures make it easier and safer for the laboratory technician to work with contaminants, infected animals and micro-organisms, toxic chemicals, poisonous and radioactive substances and live viruses. Stainless steel construction features crevice-free surfaces and rounded corners for ease of cleaning and decontamination.

▲ MICRO-BIOLOGICAL SAFETY CABINET

with micro-biological filter canister

● Filter canister may be back or top-mounted. Cabinet equipped with fluorescent lamps, cold cathode sterile light, service connections, electrical panel box with switches. Safety glass viewing window hinged to sloping front. Available in either 36" or 48" lengths. Other sizes, if desired. Air lock and stand optional. For complete description, send for Bulletin A-6.

BIOLOGICAL ENCLOSURE ▶

with refrigerated centrifuge and micro-biological filter canister

● Centrifuge is bottom-mounted, filter canister either back or top-mounted. Access openings at either end. Interior of hood has approximately ten square feet working space. Cup sink at bottom of hood. Unit equipped with UV and fluorescent lights, services for electricity, hot and cold water and three additional connections. Send for Bulletin A-7.

Standard units available, or we fabricate to your specifications. Ask us about other types of equipment in which you are interested.

Blickman-Built
STAINLESS STEEL LABORATORY EQUIPMENT

FUME HOODS
DRY BOXES
ANIMAL CAGES
PROCESS UNITS
PEG BOARDS

SEMI-HOT FUME HOODS
LABORATORY TABLES
DRY WASTE CONTAINERS
LIQUID WASTE CONTAINERS
LABORATORY STOOLS
STAINLESS STEEL SINK UNITS

ENCLOSURES
SHELVING
CABINETS
CASEWORK
TRAYS

S. BLICKMAN, INC. 6409 Gregory Avenue, Weehawken, N. J.

EDIN *research equipment gives you more benefits...*

- Accuracy
- Reliability
- Simplified Operation
- Easier Servicing
- Flexibility

Superior Oscillograph Research Consolette

You can make simultaneous recordings from a variety of sensing elements: EEG, EKG, blood pressure, skin temperature, respiration, etc., with a single Edin consolette. Modular unit handles up to eight channels of data inputs to preamps mounted in control panel. Power amplifier chassis are out of the way in the lower section of the consolette.

A Wide Choice of Amplifiers

SPECIFICATIONS

Type	Model	Gain*	Response	Noise Level RMS*
Gain DC	5238	5,000	DC-5K	10
Gain DC	5231	125	DC-5K	50
...nser Coupled	8234	10,000	1-3K	20
	5234	10,000	1-100	10
Gain CC	8235	500,000	1-3K	5
	5235	500	1-60	2
...lator	8236	20,000	DC-60	20
...ure	8241	20,000	DC-60	20
...lized DC	8239	15,000	DC-3K	20
...r	8237	1,000,000	DC-500	5

** includes power amplifier*

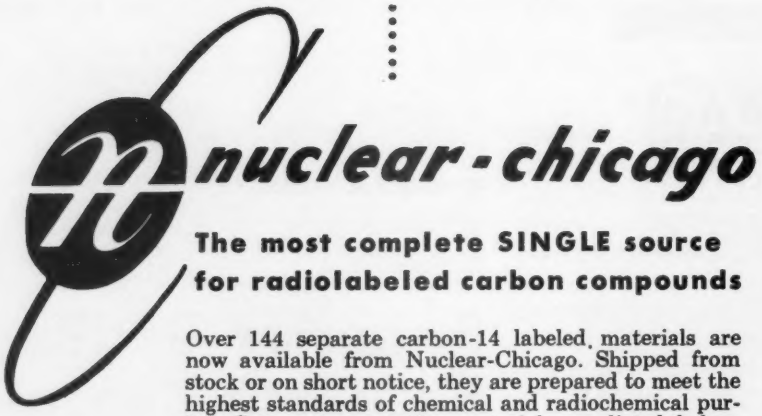
... and its perfect working partner ... the new

Waveform Frequency Analyzer

Provides automatic, continuous analysis of complex wave forms, especially valuable for EEG analysis. Bandwidth requires no adjustment nor recalibration, once set at factory. Complete range from 0.5 to 20,000 cps is available. Readout is an oscillograph recorder providing a permanent, highly legible graph record.

- Eliminates laborious, impractical arithmetical processes in analyzing frequencies.
- Plug-in filters provide unusual flexibility in choosing frequency bands.
- Ideal for analyzing data processed by the research or EEG recorders.
- Simplified Operation: only incoming voltage gain, pen excursion, and selector switches require setting.

Write for free, illustrated literature on Edin scientific and industrial research equipment.



The most complete SINGLE source for radiolabeled carbon compounds

Over 144 separate carbon-14 labeled, materials are now available from Nuclear-Chicago. Shipped from stock or on short notice, they are prepared to meet the highest standards of chemical and radiochemical purity. Some representative materials are listed below.

CARBOHYDRATES
D-Glucose-C-14
D-Glucose-1-C-14
D-Fructose-C-14
D-Mannose-1-C-14
Starch-C-14 (tobacco)

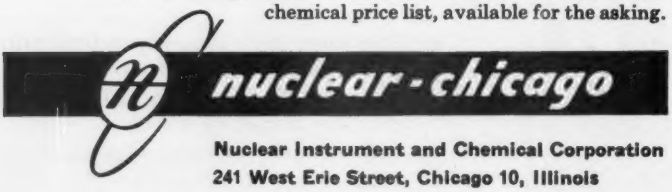
AMINO ACIDS
Glycine-1 and -2-C-14
DL-Valine-4-C-14
DL-Tyrosine-2-C-14
DL-3-(3, 4-Dihydroxyphenyl)alanine-2-C-14
L-*iso*Leucine-C-14
L-Glutamic-C-14 acid
L-Aspartic-C-14 acid
L-Lysine-C-14
L-Serine-C-14
L-Arginine-C-14
L-Valine-C-14

FATS AND FATTY ACIDS
Tripalmitin-1-C-14
Propionic-2-C-14 acid
Octanic-1-C-14 acid
Linoleic-1-C-14 acid
Myristic-1-C-14 acid

METABOLIC INTERMEDIATES
Oxalic-C-14 acid
Succinic-1-C-14 acid
Fumaric-1-C-14 acid
Pyruvic-2 and -3-C-14 acids
DL-Lactic-1 and -2-C-14 acids

MISCELLANEOUS
Adenine-8-C-14
Guanine-8-C-14
Isonicotinic (carbonyl-C-14)hydrazide
Diethyl (malonate-2-C-14)
Lauryl-1-C-14 alcohol

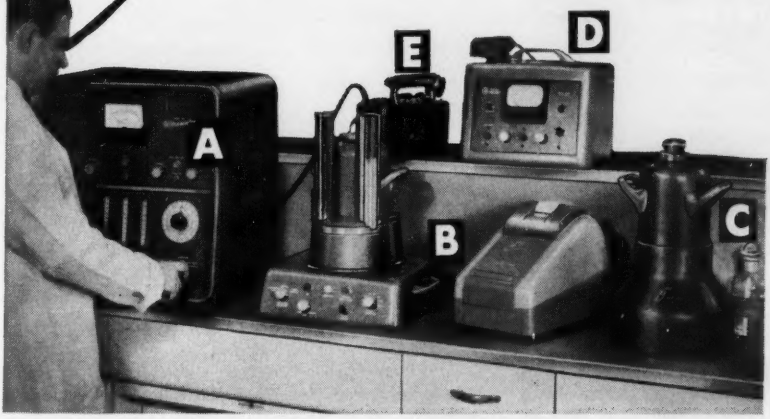
We are always happy to consider your requirements for labeled compounds not already listed on our new radiochemical price list, available for the asking.



Nuclear Instrument and Chemical Corporation
241 West Erie Street, Chicago 10, Illinois

nuclear-chicago

The ONE source for ALL your nuclear instrument and accessory needs



This radiation counting laboratory includes: A—Scaler, B—Automatic Sample Changer, C—Scintillation Well Counter, D—Count Rate Meter, E—Portable Survey Meter, all made by Nuclear-Chicago.

IT'S SOUND RESEARCH PRACTICE to be sure that the radiation measuring instruments and accessories you use are correctly coordinated to give you maximum efficiency and best laboratory performance. That's why Nuclear-Chicago offers you a complete line of nuclear equipment—instruments matched for maximum versatility and accessories developed for perfect integration with them.

New! Catalog P describes our complete line of radiation measuring instruments, radiochemicals and accessories. Write for your copy today.

nuclear-chicago

241 WEST ERIE STREET
CHICAGO 10, ILLINOIS

NUCLEAR-CHICAGO 241
241 West Erie St., Chicago 10, Illinois

Please send me Catalog P describing radiation measuring instruments, radiochemicals and accessories.

*Name*_____

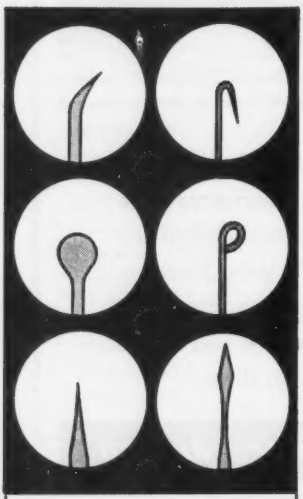
*Firm*_____

*Address*_____

New Binocular de Fonbrune Micro-forge

Extends potential application of any micromanipulator

Micro-probes, scalpels, needles and other tools made to exact specifications



Example of Micro-Instruments

The de Fonbrune micro-forge enables operator to make endless variety of tools. Pictured above are a few of the possibilities. Little practice is needed before accurate, precise micro-probes, scalpels, needles, pipettes, hooks and other tools to your exact specifications can be made.

The improved de Fonbrune micro-forge with stereoscopic microscope is an indispensable accessory for all types of micromanipulators. With it, micro-pipettes, micro-scalpels and other micro-instruments may be fabricated under controlled conditions. Little practice is necessary to acquire manipulating skill. Availability of standard and special micro-tools is a genuine advantage in utilizing the full potentialities of the micromanipulator. The micro-forge is a combination of optical and electric forge units. The stereoscopic microscope provides an erect image; has 4X paired objectives and 9X paired wide-field eyepieces, providing a magnification of 36X. Electrical forge is mounted on an adjustable stage directly in front of microscope objectives. Two insulated clamps support electrodes connected by a platinum-iridium filament, providing temperatures from about 120° F. to 3000° F. Air jets are mounted on stage. Lamp housing is set opposite objective and forge unit. A pivoting support for material is mounted at periphery of a disc in front of lamp housing. Both spindle and disc may be rotated about their own axes and the entire assembly adjusted vertically or horizontally. Controls are provided for adjusting light to objective. A detailed manual of instructions is furnished with each micro-forge. Accessory equipment including air pump, rheostats, and transformer is contained in the base.

Write for prices and descriptive bulletin T114

aloe scientific

DIVISION OF THE A. S. ALOE COMPANY

5655 Kingsbury • St. Louis 12, Missouri

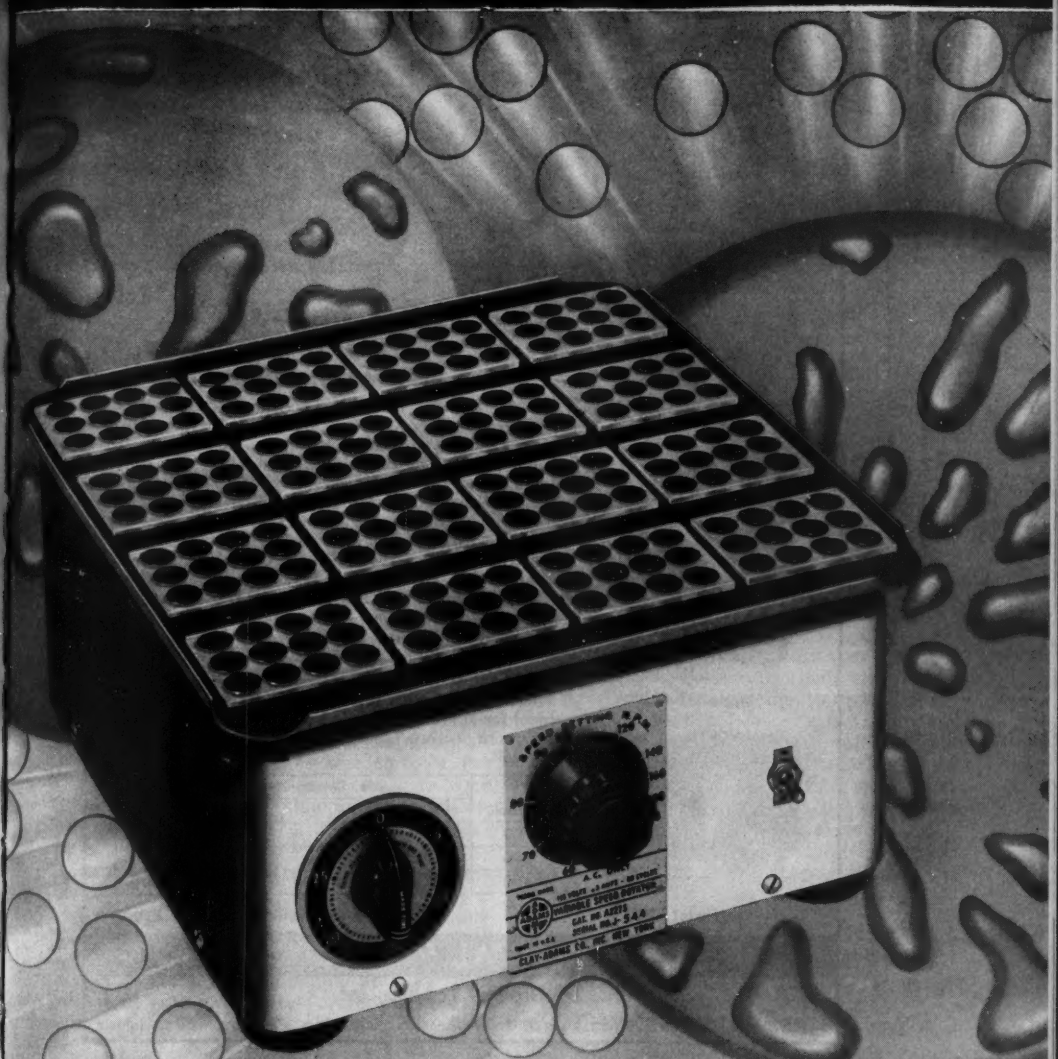
kinds of YANKEE® Rotators, to fulfill your requirements

For maximum accuracy, uniformity and control in routine procedures...YANKEE Rotators are engineered for heavy duty and will give long and efficient service.

A-2270—130 RPM
11 x 11" platform,
$69.00
(or A-2271, 180 RPM)

A-2273—AC Variable
Speed Rotator
13 x 13" platform,
60-270 RPM, $144.00

A-2272—AC-DC Variable
Speed Rotator
13 x 13" platform,
70-210 RPM, $97.50



Order from your dealer. He also stocks:
Blood Testing Kits • Gold Seal Slides and Cover Glasses • Adams Centrifuges • Yankee Pipettes Adams Laboratory Counters • Medichromes

Clay-Adams
NEW YORK 10

1956

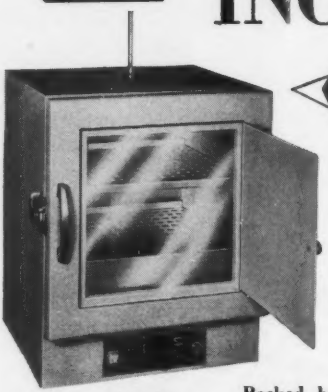
C.S.&E.
DIV OF LABLINE, INC.

Imperial SERIES

INCUBATORS

With the New Forward *"Lab Look"*

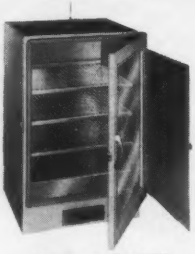
Open this door and all other incubators seem outdated!



"Imperial 200"

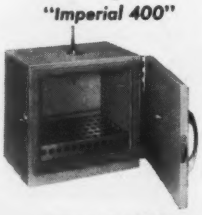
Room to 60°C
. . . for Incubation or Paraffin Embedding

Backed by 50 years of experience as America's pioneer manufacturer of constant temperature cabinets, Chicago Surgical & Electrical Company now announces its new Imperial Series for 1956. Imperial Incubators provide uniform heat because of Thermo-Wall principle—no internal heaters. Working chamber is solid aluminum, Window-Dor has glass viewing window, with hinged door to block external light. Contents may be viewed without disturbing internal temperature. Many other advanced principles of incubator construction. Remember—only C.S. & E. Thermo-Wall heating gives greatest temperature uniformity—the main requirement for best incubation. Finished in exclusive C.S. & E. Sand-Tan.



"Imperial 400"

"Imperial 100" is a budget priced incubator with Thermo-Wall heating. Temperature range to 40° C. Ideal for running multiple individual incubations or for use in doctor's laboratory. Sets on table or hangs on wall.



"Imperial 100"

Model	Inside Dimensions H.	W.	D.	Price
100	8"	9"	7"	$69.50
200	13"	13"	13"	112.50
400	27"	18"	18"	155.00

Write for Bulletin F-9

CHICAGO SURGICAL & ELECTRICAL CO.

Division of Labline, Inc.

3070-82 West Grand Avenue • Chicago 22, Illinois

NEW PYREX® brand pipettes
COLOR CODED for safe, rapid selection

Now you can pick the pipette you want by a quick glance at the color band.

You can get both Mohr and serological types with 6 different bands of color—red, yellow, blue, green, black and white. You can designate your own important meaning to each color. For example, you might assign a color to each chemist or technician. Colors could indicate sources or sizes—or the type of test—or sequence of use—or samples to be tested—or the priority of samples to be tested. And how many more ways!

Corning Color Coding makes even more outstanding the most useful pipettes you can work with. PYREX brand pipettes are designed for accuracy, readability, strength. You get them with double-beveled tips that are strong. And the tips are tapered for smooth, even delivery flow.

The measuring type conforms in design to NBS Circular C-434 Specifications, and serological designs conform in design to Federal Specifications DD-V-581a.

We believe that our touch of color will add to your laboratory's efficiency, make it easy for everyone to maintain accuracy in many ways.

Ask your laboratory supply dealer to show Corning Color Coded Pipettes to you soon.

"For Corning's full line of pipettes, see pages 102 to 109 in the new 'Laboratory Glassware Catalog' LP36."

CORNING GLASS WORKS
86-9 Crystal Street, Corning, N. Y.

Corning means research in Glass

PYREX® laboratory ware

. . . the tested tool of modern research

LEA & FEBIGER BOOKS

NEW 2nd EDITION

Soffer—Diseases of the Endocrine Glands

By Louis J. Soffer, M.D., F.A.C.P.

Clinical Professor of Medicine, State University of New York, College of Medicine in New York City

This is a "how to" book on the complete management of all clinical syndromes. The new edition is fully up to date with much new material and many new illustrations.

New 2nd Edition • 1032 Pages
102 Illustrations and 3 Plates in Color
28 Tables • $16.50

PHYSIOLOGY AND PHARMACOLOGY

Starling—Principles of Human Physiology. Edited by Sir Charles Lovatt Evans, D.Sc., F.R.C.P., F.R.S., LL.D., Birmingham; University of London. *1233 pages. 721 illustrations, some in color. New 12th edition. $12.50*

Wiggers—Physiology in Health and Disease. By Carl J. Wiggers, M.D., D.Sc., F.A.C.P., Western Reserve University, Cleveland, Ohio. *1242 pages. 280 illustrations. 5th edition. $10.00*

Grollman—Pharmacology and Therapeutics. By Arthur Grollman, Ph.D., M.D., F.A.C.P., Southwestern Medical School, University of Texas, Dallas, *866 pages. 127 illustrations, 56 tables. 2nd edition. $10.00*

PATHOLOGY AND TOXICOLOGY

Simmons and Gentzkow—Medical and Public Health Laboratory Methods. 35 Contributors. Edited by the Late James S. Simmons, M.D., Ph.D., Harvard School of Public Health; and Cleon J. Gentzkow, M.D., Ph.D., Bureau of Laboratories, of Health, Commonwealth of Pennsylvania. *1191 pages. 115 illus. and 9 plates in color. 129 tables. New 6th edition. $18.50*

Levinson and MacFate—Clinical Laboratory Diagnosis. By Samuel A. Levinson, M.D., Ph.D., University of Illinois College of Medicine; and Robert P. MacFate, Ch.E. M.S., Ph.D.; Chief, Division of Laboratories, Board of Health, City of Chicago. 5 Contributors. *1248 pages. 244 illustrations and 13 plates, 11 in color. 142 tables. New 5th edition. $12.50*

Herbut—Pathology. By Peter A. Herbut, M.D., Jefferson Medical College Hospital, Philadelphia, Pa. *1227 pages, 7" x 10". 1378 illustrations on 651 figures and 6 plates in color. $16.00*

Thienes and Haley—Clinical Toxicology. By Clinton H. Thienes, M.D., Ph.D., University of Southern California; and Thomas J. Haley, Ph.D., University of California at Los Angeles. *457 pages. Illustrated. 33 tables. 3rd edition. $6.50*

BIOCHEMISTRY AND NUTRITION

Hardy—Fluid Therapy. By James D. Hardy, M.S. (Chem.), M.D., F.A.C.S., University of Mississippi, Jackson. *255 pages. 77 illustrations. 8 tables. $5.50*

Wohl and Goodhart—Modern Nutrition in Health and Disease: Dietotherapy. *55* Contributors. Edited by Michael G. Wohl, M.D., Hahnemann Medical College and Hospital, Philadelphia, Pa.; and Robert S. Goodhart, M.D., Columbia University School of Public Health, New York. *1062 pages. 80 illustrations. 127 tables. New. $18.50*

LEA & FEBIGER WASHINGTON SQUARE PHILADELPHIA 6, PA.

Please enter my order and send me books listed below.

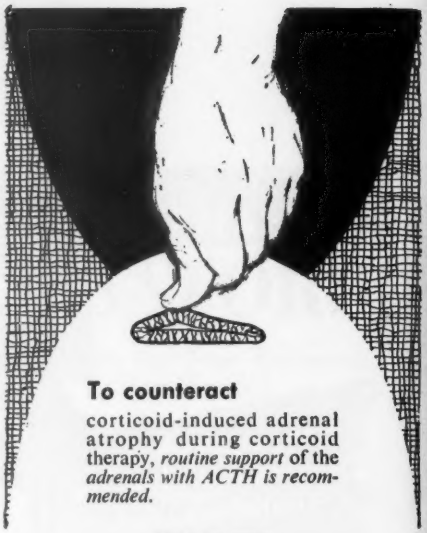
☐ Check enclosed ☐ Bill me ☐ Charge my account

NAME_____

ADDRESS_____

CITY_____ ZONE___ STATE_____

FP 9-56



To counteract
corticoid-induced adrenal atrophy during corticoid therapy, *routine support* of the *adrenals with ACTH is recommended.*

THIS IS THE PROTECTIVE DOSAGE RECOMMENDATION FOR COMBINED CORTICOID-ACTH THERAPY

- When using *prednisone* or *prednisolone:* for every 100 mg. given, inject approximately 100 to 120 units of HP* ACTHAR *Gel.*
- When using *hydrocortisone:* for every 200 to 300 mg. given, inject approximately 100 units of HP* ACTHAR *Gel.*
- When using *cortisone:* for every 400 mg. given, inject approximately 100 units of HP*ACTHAR *Gel.*

Discontinue administration of corticoids on the day of the HP*ACTHAR *Gel* injection.

HP*ACTHAR® *Gel*
(IN GELATIN)

The Armour Laboratories brand of purified adrenocorticotropic hormone—corticotropin (ACTH)

*Highly Purified

Unsurpassed in Safety and Efficacy
More than 42,000,000 doses of ACTH have been given

THE ARMOUR LABORATORIES
A DIVISION OF ARMOUR AND COMPANY
KANKAKEE ILLINOIS

International

centrifuge model CM

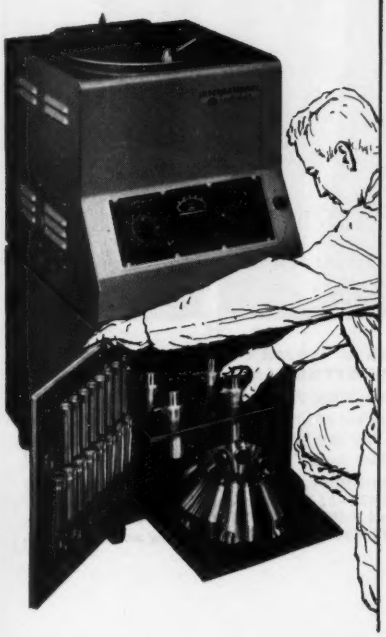
. . . for routine centrifuging

Designed for the moderate budget, the Model CM is a heavy-duty Centrifuge for all ordinary applications where very high speeds are not essential. It is an unusually versatile unit with interchangeable analytical heads for spinning tubes and bottles in the angle and horizontal positions and basket style heads for filtration and clarification.

EMPHASIS ON FUNCTIONAL EFFICIENCY

All operating units are completely enclosed in a handsomely modern functional cabinet of two piece construction, which incorporates shelves for extra heads and features International's new "Stor-a-Door" for storing shields and trunnion rings. Controls are easily accessible and indicators are on eye-level angle for utmost efficiency.

. . . with Emphasis on Functional Efficiency



NEW IMPORTANT FEATURES

Stainless Steel Guard Bowl, corrosion resistant, easy to clean, no paint to chip or peel.

Finger Touch Brake, for safe, smooth deceleration.

Autotransformer, stepless speed control without heating.

Electric Tachometer, non-mechanical continuous speed indication.

Permanent Speed and Force Table, for quick reference.

Two-Piece Construction, for use as bench-Model Centrifuge when desired.

SPECIFICATIONS

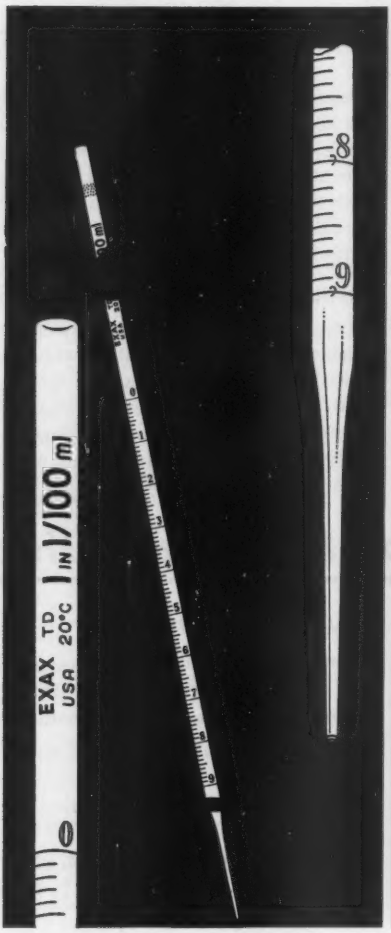
Speed — 1200 to 4500 r.p.m. — 290 to 3200 G.
Capacity — 15, 50, 100 ml. tubes; 250 ml. bottles; 3 liter baskets.
Height — 39″, **Width** — 20″, **Depth** — 23″.

International Equipment Co.

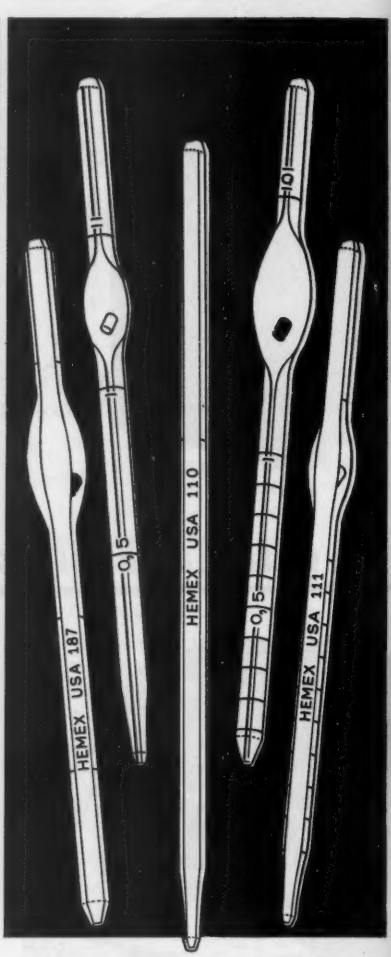
1284 SOLDIERS FIELD ROAD • BOSTON 35, MASSACHUSETTS

NEW KIMBLE

designed for specific needs—longer



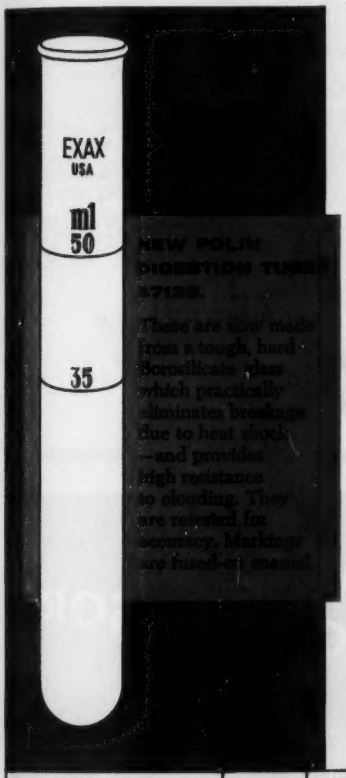
KIMBLE LONG TIP PIPETTES. Permit easy manipulation in small openings. 37022 is a measuring style graduated to a base line. 37039 is a blow-out, serological style graduated to the tip. They are made of Borosilicate glass with a high chemical resistance that prevents clouding . . . markings are permanent . . . individually retested for accuracy.



NEW HEMEX BLOOD DILUTING PIPETTES. A *new* line of quality pipettes providing National Bureau of Standards accuracy at *low cost*. Markings are permanent. They can be removed only by dissolving the glass itself. Tubing and mouthpiece are sold separately, for added economy. **#36550 and 36555—11-line for red and white corpuscles. #36560 and #36565—3-line for red and white corpuscles. #36650—Sahli. #36655—tubing and mouthpiece.**

ITEMS

life—greater convenience and economy



✓ GRADUATION LINES AND NUMBERS ARE PERMANENT.
The fused-in colored material used in the lines and numbers can be removed only by dissolving the glass itself.

✓ GLASS WITH EXCEPTIONAL RESISTANCE TO CLOUDING.
Kimble Borosilicate glass has a higher chemical resistance than any other glass available. This means longer life with sparkling clarity and resultant savings on replacements.

✓ ACCURACY INSURED BY RIGID SPECIFICATIONS.

✓ THOROUGHLY ANNEALED FOR GREATER STRENGTH.
Proper annealing provides greater mechanical strength.

✓ UNIFORMITY OF PRODUCT ASSURED THROUGH QUALITY CONTROL.
Careful control in every manufacturing stage assures product uniformity day in and day out.

DON'T ACCEPT SUBSTITUTES.

If your dealer cannot supply Kimble pipettes and tubes—write: Kimble Glass Company, subsidiary of Owens-Illinois, Toledo 1, Ohio.

Description and Catalog Number	Capacity ml.	Subdivision ml.	Qty. In Case	Description and Catalog Number	Qty. In Case
Mohr Measuring Pipettes 37022 with long tip	0.1	1/100	12	Hemex Blood Diluting Pipettes:	
	0.2	1/100	12		
	0.5	1/100	12	36550—11 line, red	36
	1	1/100	12	36555—11 line, white	36
	2	1/10	12	36560—3 line, red	36
	5	1/10	12	36565—3 line, white	36
Serological Pipettes 37039 with long tip	0.2	1/100	12	36650 -Sahli	36
	1	1/100	12	36655 Rubber Tubing and Mouthpiece	72
	5	1/10	12		
47125 Folin Digestion Tubes	50		36	There is no substitute for Kimble quality.	

KIMBLE LABORATORY GLASSWARE
AN (O-I) PRODUCT

OWENS-ILLINOIS
GENERAL OFFICES • TOLEDO 1, OHIO

NEW ENGLAND NUCLEAR RADIOACTIVE STEROIDS

High specific activity labeled steroids, 5–15 microcuries per milligram . . . purified chromatographically . . . available from stock.

	Price per microcurie			
	5–99	100–499	500–999	1000 and over
• Cholesterol-4-C^{14}	$ 8.00	$6.00	$4.50	$3.00
• Cholestenone-4-C^{14}	7.00	5.00	3.50	2.50
• Progesterone-4-C^{14}	10.00	7.50	5.50	4.00
• Testosterone-4-C^{14}	5.00	3.50	2.50	2.00
• Desoxycorticosterone-4-C^{14} Acetate	$20.00			

Write for Complete Radiochemical Catalog

new england nuclear corp.®
575 ALBANY STREET, BOSTON 18.

For the first time a complete

"LUNG FUNCTION STATION"

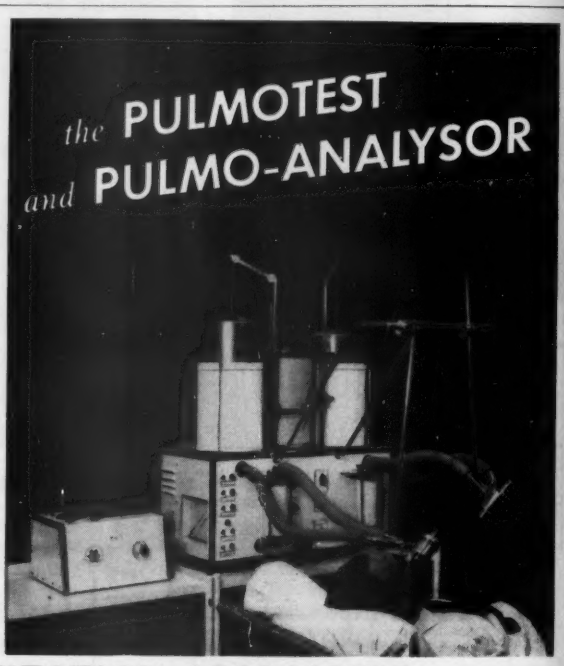
in one complete integrated unit

CLINICAL APPLICATIONS

- Closed circuit technique for measurement of Functional Residual Capacity and Residual Volume using Helium or Oxygen.
- Vital Capacity, Inspiratory and Expiratory Reserve Volumes
- Timed Vital Capacity
- Maximum Breathing Capacity
- Oxygen Consumption: Basal Metabolic Rate
- Bronchospirometry: Oxygen consumption and Vital Capacity of each lung
- CO_2 concentration of expired gases

All tests performed in room air

Special models available for infants and small animals.



Send for complete brochure from:

INSTRUMENTATION ASSOCIATES
17 West 60th Street • New York 23, N. Y.
Exclusive representative in the United States and South America

Flight muscle from wing of bumble bee. Magnification: 51,000X.

Delbert E. Philpott, Microscopist, Marine Biological Laboratory.

Microscopist "muscles-in" on bee's flight secrets . . .
aided by RCA Electron Microscope

The flight of the bumble bee long has been under scrutiny—especially by aerodynamicists—but it took a microscopist to delve beyond the surface into the muscle structure. At Woods Hole, Mass., Delbert E. Philpott of the Marine Biological Laboratory has been studying muscles from bees' wings. He says: "The tremendous increase in resolving power made possible with the RCA Electron Microscope has allowed visual observation of the structural details in muscle which were impossible to see by any other method. Certainly our knowledge of muscle has been advanced as much in the last decade with RCA Electron Microscope as it was previously advanced with the light microscope. That's real progress."

RCA Electron Microscopes have opened many new avenues of research in the fields of chemistry, metallurgy, medicine, bacteriology and biology. Magnification of the RCA Electron Microscopes is variable in steps from 1400 to 30,000 diameters, with useful photographic enlargements up to 300,000 diameters. Thus, objects whose very existence could previously only be surmised have become visible to the naked eye.

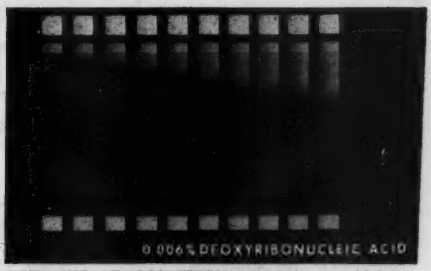
Why not find out how these wonderful instruments can be of service to you? Installation supervision is supplied and contract service by RCA Service Company is available, if desired.

For further information on the use of the RCA Electron Microscope, write to Dept. 000, Building 15-1, Camden, N.J. In Canada: RCA VICTOR Company Limited, Montreal.

RADIO CORPORATION of AMERICA
CAMDEN, N. J.

HERE

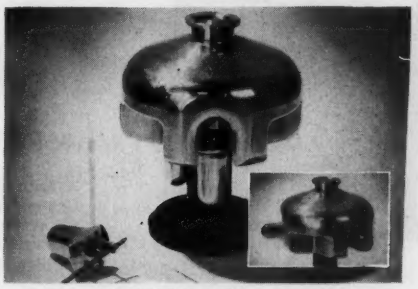
RAYLEIGH INTERFERENCE FRINGES provide high-sensitivity measurement of solute concentration at any level in analytical cell. Used for determining molecular weights—both high and low—by the equilibrium method, as well as for measuring sedimentation velocity by the centrifugal transport of material past a given level in the cell.



ULTRAVIOLET ABSORPTION method gives very high sensitivity with respect to concentration rather than concentration gradient, as in schlieren techniques. Newly-designed optical system is particularly valuable for such materials as nucleoproteins and other ultraviolet-absorbing substances exhibiting diffuse or inhomogeneous boundaries.

HOW new developments have increased the versatility of Analytical Ultracentrifugation

SYNTHETIC-BOUNDARY cell permits establishment of an immediately-measurable boundary by a layering technique. This extends sedimentation-rate measurements from the previous practical lower molecular-weight limit of 10,000, downward to 500.



40,000-rpm SWINGING-BUCKET rotors make preparative operations most closely comparable with analytical, give most accurate estimation of sedimentation by sampling. Boundaries sediment normally and undergo minimum reorientation. Quartz inserts permit spectrography.

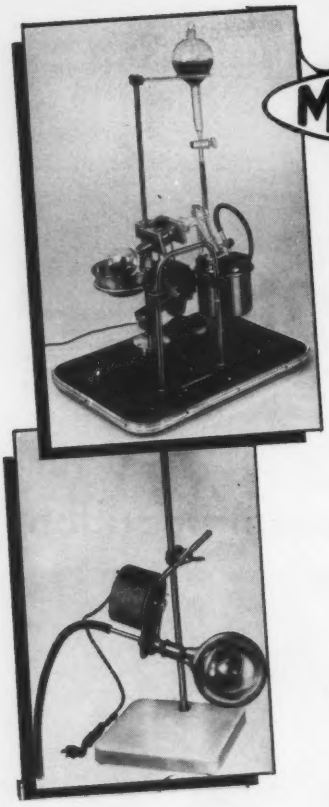
Since 1947, when it was introduced, the Spinco Model E Analytical Ultracentrifuge has been constantly broadened in utility by new developments in equipment and technique.

Besides the specific items illustrated above, provisions have been made for: (1) simultaneous analysis of two materials in the same rotor; (2) temperature measurement and continuous, automatic control of operating rotors to better than 0.1 degree C from zero to 100 degrees C; and many other specialized functions.

Each of these developments has been engineered to permit its application to any Spinco Model E in existence. Your inquiry is invited for further information on these or other developments which may provide solutions to your problems in physical research.

Spinco *division*

BECKMAN INSTRUMENTS, INC.
BELMONT CALIFORNIA



MACHLETT *Automatic Distillation Apparatus*

✶ ROTOVAP

Suitable for batch or continuous operation, Rotovap is completely automatic and provides safe, rapid and efficient evaporation of either dilute or concentrated solutions. Rotovap will distill 100 ml. of aqueous solution at 55°C. in 10 to 15 minutes under the reduced pressure of an ordinary water aspirator. Its speed for non aqueous solutions is correspondingly high.

Due to the functional design of Rotovap, evaporator flask can be easily heated with a water bath or infra-red lamp. The condenser flask can be cooled with a special water spray provided with Rotovap. For work with heat-sensitive compounds, condenser flask may be cooled with ice.

For detailed information, write for folder RV.

32-922 Rotovap complete as illustrated but without funnel, ring and clamp $185.00
Self Lubricating Teflon Ball and Socket Assembly where all glass system is not required

32-922TB Teflon Connecting Spindle $9.75

32-922TC Teflon Connecting Elbow $11.50

✶ FLASH-VAC

As with Rotovap, Flash-Vac utilizes the principle of spreading a thin film of solution over a large area by rapidly rotating—60 R.P.M.—the evaporating flask and subjecting the flask to vacuum. When heat is desired on the evaporation flask, an infra-red lamp may be mounted on rear support rod or a water bath may be used.

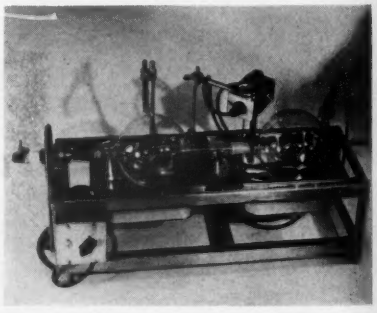
For details write for Bulletin F. V.

32-922FV Flash-Vac complete with 18-8 stainless steel sleeve with 24/40 taper, Vacuum tube, "O" ring seals, enclosed motor, support rod and clamp. Flask and ring stand optional $96.50

✶ FLASH-EVAPORATOR

Available in two models FE-2 for batch operation only and FE-2C for both batch and continuous operation, Flash-Evaporator has an evaporator flask on the right hand side which revolves in a hot water bath and a condenser flask on the left which is simultaneously cooled by a stream of cold water or ice. The two rotating flasks are connected to the revolving shaft by a standard taper joint.

For details write for Bulletin FE



32-922FE-2 for batch evaporation complete motor, rheostat, 500 watt heater, 2 liter evaporator and condenser flasks with T/S 24/40 but without thermo regulator $215.00

32-922FE-2C for continuous or batch operation complete with motor rheostat, 500 watt heater, 2 liter evaporator and condenser flasks with standard tapered joints, thermo-regulator and complete glassware for batch operation $330.00

DELIVERIES FROM STOCK

MACHLETT

TELEPHONES
NEW YORK
LExington 2-1311
NEW JERSEY
Elizabeth 4-1330

E. MACHLETT & SON
ESTABLISHED 1915
A COMPLETE SERVICE FOR THE LABORATORY
APPARATUS • INSTRUMENTS • CHEMICALS • GLASS BLOWING
220 EAST 23rd STREET • NEW YORK 10, N. Y.

EMSON

CABLE
EMSONGLAS
•
TELETYPE
N. Y. 1-2444

A new use for

Schwarz

N-Ethyl Maleimide (NEM)

for specific
detection of
SH compounds
and
thiol esters

•

simple
sensitive

•

It is now possible to detect and distinguish sulfhydryl compounds by a color reaction with NEM. The reagent is particularly useful for developing paper chromatograms. Details of the procedure appear in Science 123, 981 (June 1, 1956).

Among Schwarz products giving positive color reaction with NEM are:

Glutathione
***S*-Acetyl Glutathione**
DL-Homocysteine Thiolactone • HCl
***N*-Acetyl DL-Homocysteine Thiolactone**
L-Cysteine • HCl • H_2O
Cysteinyl Glycine
Thiouracil

Write for further information

SCHWARZ LABORATORIES, INC.

Leading Manufacturers of Sulfhydryl Reagents and Yeast Biochemicals

230 WASHINGTON STREET, MOUNT VERNON, NEW YORK SL 370A

NEW (2nd) EDITION

~ SODEMAN—Pathologic Physiology

In line with modern concepts this *New (2nd) Edition* shows what happens to the functioning of the body in the presence of disease and *why* and *how* disease symptoms occur. The book bridges the gap between physiology and medicine by presenting a clinical picture of disease as physiologic dysfunction. New chapters on genetics, growth and neoplasia and on the nervous system round out the coverage of the book. The completely rewritten section on diabetes is now a brilliant exposition of the subject. You will also find much modern help on disorders of every part of the body—blood—heart—lungs—stomach—intestines—bones—endocrine glands—etc.

By 29 collaborators, edited by WILLIAM A. SODEMAN, M.D., F.A.C.P., Professor of Medicine and Chairman of the Department of Medicine, School of Medicine, University of Missouri, Columbia, Missouri. 963 pages, 6½" x 9¼", with 173 illustrations. $13.00. *New (2nd) Edition!*

NEW

~ SPECTOR—Handbook of Toxicology

Acute Toxicities of Solids, Liquids and Gases in Laboratory Animals is the first of a 5 volume work sponsored by the National Research Council. When complete it will be the *most comprehensive collection of data ever compiled* on the toxic qualities and dosages of all substances which have known adverse effects on living organisms. In this first volume, 2363 chemical compounds are listed, and the lethal dosages of each in various laboratory animals are indicated. Information about the variation of toxicity is included—depending upon size of dose, method of administration, rate of absorption, site of injection, etc. Original sources of tabular data are given.

Edited by WILLIAM S. SPECTOR. Prepared under the direction of the Committee on the Handbook of Biological Data, Division of Biology and Agriculture, National Academy of Sciences—The National Research Council. Principal Contributor—WOLFGANG F. VON OETTINGEN, M.D., Ph.D., Chief Toxicologist, The National Institutes of Health, Bethesda, Maryland.
Volume I, 408 pages, 8½" x 11". $7.00. (4 more volumes to be published). *New!*

NEW (2nd) EDITION

~ WELLS—Clinical Pathology

For the *New (2nd) Edition*, this popular book has been completely rewritten to reflect the many important changes made in laboratory methods and basic physiology during the past five years. It explains in a clear, understandable manner when laboratory tests may be useful in making or confirming a diagnosis. It tells exactly which tests should be utilized, interprets what the test findings may signify in terms of disease, and offers thoughtful advice on the possible inadequacies of and sources of error in each test. A new chapter on laboratory methods describes the most readily available tests for making studies of blood, urine, feces, etc.

By BENJAMIN B. WELLS, M.D., Director of Clinical Investigation, the Lynn Clinic, Detroit. Formerly Professor of Medicine and Head, Department of Medicine, Creighton University, Omaha, Nebraska. About 540 pages, 6" x 9¼", illustrated. *Just Ready!*

W. B. SAUNDERS COMPANY
West Washington Square Philadelphia 5
London: 7 Grape Street

the new Autotechnicon® takes a third less space has 20% more capacity

actual size on 10 foot bench

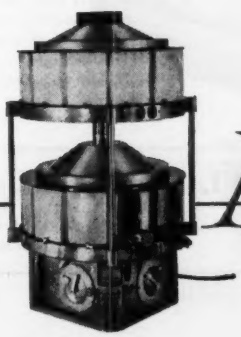
From the very beginning . . . in the very first Autotechnicon which introduced automation to tissue-processing . . . a round deck was used because only in a circle can you condense so many beakers, so compactly. It's simple geometry.

But even the best idea can be improved, the deck is still a perfect circle . . . but it is smaller by a third. Still, the new radial beakers have 20% more capacity of both fluid and tissues than the previous ones.

Since it doesn't sprawl all over the place, as you see above, the New Autotechnicon, occupies only the very corner of your lab-bench, leaving almost the entire bench free and clear for other work.

the entire Autotechnicon is mounted on a free-wheeling quiet turntable . . . just rotate it to bring any beaker to the front

Write today for Booklet 2-AT, for description of these new instruments.



the Autotechnicon®

Trailblazer in histologic automation

THE TECHNICON COMPANY

Chauncey New York

Paris Office: COMPAGNIE TECHNICON • 7 rue Georges Ville • Paris

We are prepared to consider requests from professionally qualified investigators for experimental quantities of vitamin E in the form of *d*-alpha-tocopherol or its derivatives. Address inquiries to: Dr. Norris D. Embree, Director of Research, ***Distillation Products Industries,*** Rochester 3, N. Y. (Division of Eastman Kodak Company).

DPi *supplier of bulk tocopherols to the pharmaceutical industry*

For Immediate Shipment

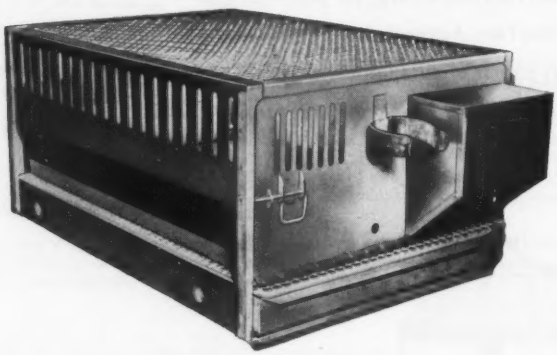
ALL Chemicals
ALL Drugs
ALL Reagents
ALL Pharmaceuticals

Ask for our new complete catalog

BIOS *Laboratories, Inc.*
17 West 60th St. New York 23 N. Y.
Plaza 7-8171

ANIMAL CAGES AND RACKS
LABORATORY EQUIPMENT

Quality Cages Since 1912

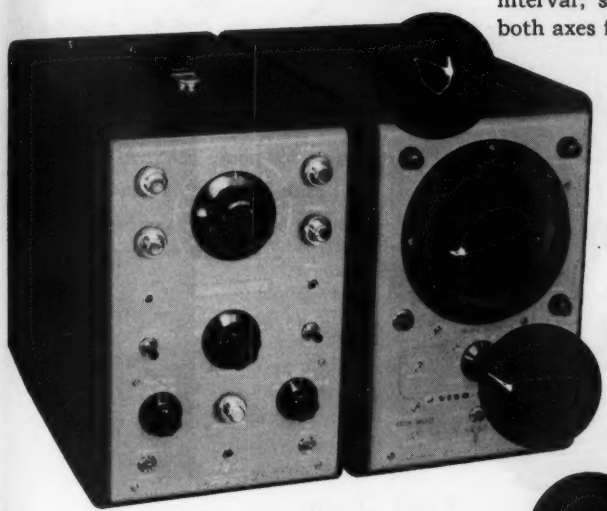


Write for Catalog "P"

ACME SHEET METAL WORKS

1121 EAST 55th STREET • CHICAGO 15, ILLINOIS

FOR VIEWING EITHER VECTOR LOOPS OR REGULAR ECG LEADS



The SANBORN Vector Amplifier displays cube or tetrahedral vector leads — or ECG leads — on Viso-Scope screen

Specifically designed for use with the Sanborn Viso-Scope oscilloscope, this new Vector Amplifier provides complete and simplified control for all vectorcardiography functions. Any of 13 lead derivations are selected by turning a single knob. Other adjustments include control of trace width, height and position, frequency response and timing interval; selection of mirror images of both axes for photography; introduction of 1 mv standardization signal; beam intensity (Z axis) control.

In use, the vector loop or ECG complex appears as a sharply defined trace of long-persistence on the Viso-Scope screen. To photograph the image, a Fairchild, DuMont or other camera may be mounted on the Viso-Scope bezel.

BASIC FEATURES

Model 185 Vector Amplifier

LEADS
ECG: 1, 2, 3, AVR, AVL, AVF, V and STD position
TETRAHEDRON SYSTEM: Frontal, Sagittal and Horizontal
CUBE SYSTEM: Frontal, Sagittal, Horizontal
STD for simultaneous X and Y axis calibration for both tetrahedron and cube systems
SENSITIVITY: (Basic) 10" per mv
PATIENT CONNECTION: 9 wire cable (4 tips marked for cube vectorcardiography) and 9 electrodes furnished
SIZE, WEIGHT: Compact case 13⅞" x 9½" x 19⅞"; weight 28 lbs.

Complete descriptive and operating data available on request.

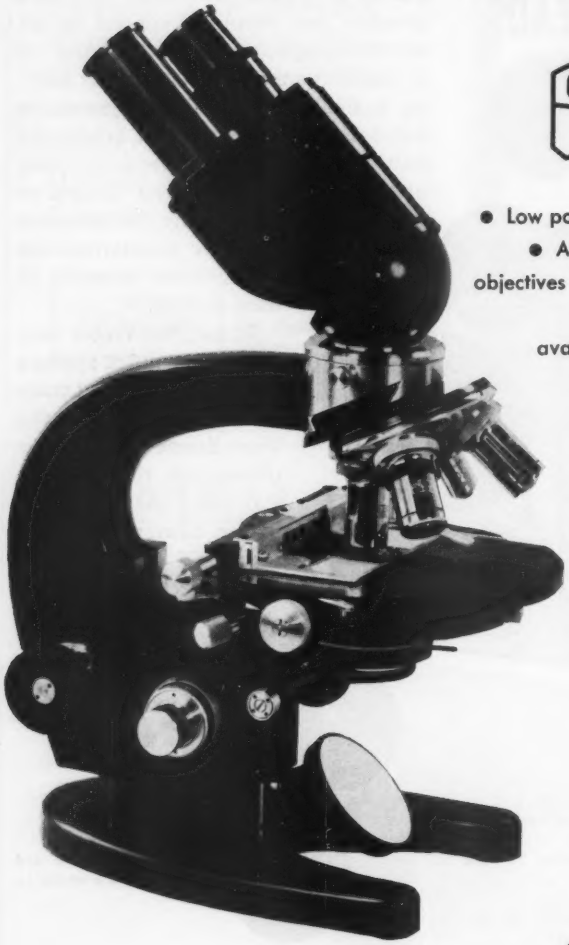
The Model 179 Electronic Switch and Model 169A Viso-Scope are products of the same designers and makers as the well-known Viso-Cardiette, direct writing electrocardiograph.

SANBORN COMPANY

CAMBRIDGE 39, MASSACHUSETTS

FOR THE DISCRIMINATING MICROSCOPIST
THE Lg MICROSCOPE STAND

AN IDEAL LABORATORY OR RESEARCH MICROSCOPE FEATURING THE WELL-KNOWN CARL ZEISS, JENA PRECISION AND OPTICAL QUALITIES



CARL ZEISS JENA

- Low positioned coarse and fine adjustments
- Available with achromat or apochromat objectives
- Extreme versatility, accessories available for phase contrast, polarizing, dark field and photomicrography
- Selection of large square or circular mechanical stages

For further information on this and other Carl Zeiss, Jena instruments, consult your dealer, or write to

Scientific Instrument Division

ERCONA CORPORATION 551 Fifth Avenue, New York 17, N. Y.

Exclusive American Representative for VEB Carl Zeiss, Jena

The Proceedings of the World Health Organization's Seminars held at Leyden and Stockholm in October - November, 1950, have just been published:

Infant Metabolism

The seminars promoted an exchange of experience and research data among pediatricians and biochemists in the important field of physiological and pathological metabolism in children. Current physiological, biochemical, nutritional and clinical problems were discussed.

Outstanding representatives of two continents in chemistry, biochemistry, physical chemistry, nuclear physics and physiology were contributors — including three Nobel prize winners: Dam, Hevesy, and Tiselius. The results of many of the research projects have not been published till now. *Infant Metabolism* serves as a comprehensive single reference source of data which are now scattered in numerous publications from many countries. Some of the topics are water and electrolyte metabolism; metal and mineral metabolism; protein and vitamin metabolism; isotopes in metabolism; evaluation of nutritive status; metabolism in premature and newborn infants.

457 pages illustrated $8.00

Ready Now!

New 2nd edition

TEXTBOOK OF BIOPHYSICAL CHEMISTRY

(formerly Physical Chemistry for Students of Biochemistry and Medicine)

By Edward Staunton West, Ph.D.,
Professor of Biochemistry, University of Oregon Medical School

Because application of principles is discussed, the student is able to see the physical chemical principles in physiological operation. The Brönsted concept simplifies biochemical problems such as acid-base balance. The treatment of isotopes particularly suited to tracer studies has been expanded.

499 pages illustrated Probably $6.00

from your bookstore or

The Macmillan Company
60 FIFTH AVENUE, NEW YORK 11, N.Y.

Distributors in Canada: BRETT-MACMILLAN LTD., 25 Hollinger Road, Toronto

AAAS New York Meeting

DECEMBER 26-31, 1956

The 123rd Meeting of the American Association for the Advancement of Science will include sessions of 18 AAAS sections and of some 84 participating organizations. Programs of particular interest to members of the Federation are:

I. **AAAS.** Presidential Address by George W. Beadle, **Dec. 28**; general symposium—Moving Frontiers of Science, **Dec. 27, 28.**

II. **AAAS Section C—Chemistry.** Contributed papers in organic and medicinal chemistry, **Dec. 26**; symposium on Substances with Selective Toxicity, arranged by Werner Braun, **Dec. 27**; symposium on Steroids, arranged by Boyd Woodruff, **Dec. 28**; symposium on Organic Chemistry, arranged by Ellis Brown, **Dec. 29.**

III. **AAAS Section F—Zoological Sciences.** Symposium, joint with the Genetics Society of America, on The Biochemistry of the Cell Nucleus, arranged by A. W. Pollister, **Dec. 28**; two-session symposium on Problems of Aging in Plants and Animals, arranged by Howard J. Curtis, Paul J. Kramer and Conrad G. Mueller, **Dec. 29.**

IV. **AAAS Section G—Botanical Sciences.** Symposium on Some Unsolved Problems in Biology, arranged by Barry Commoner, **Dec. 28.**

V. **AAAS Section I—Psychology.** Invited papers on Experimental Psychopathology, Physiological Mechanisms of Motivation and Reward, and Sensory Functions, arranged by Conrad G. Mueller, **Dec. 26, 28, 29.**

VI. **AAAS Section N—Medical Sciences.** Four-session symposium on Evolution of Nervous Control from Amoeba to Man, ranging from the rudiments of intracellular phenomena in protozoans through intercellular communication and neurohumors in both invertebrates and vertebrates to the basic actions of drugs on animal and human behavior, arranged by Bernard B. Brodie, **Dec. 29, 30.** Included will be the Vice Presidential Address of Irvine H. Page; twelfth presentation of Theobald Smith Award; second presentation of Anne Frankel Rosenthal Memorial Award for Cancer Research and first presentation of Richard and Hinda Rosenthal Prize for Research on Cardiovascular Problems.

VII. **AAAS Section Nd—Dentistry, American Dental Association, et al.** Symposia on Forensic Dentistry, arranged by W. M. Krogman, and on Antienzymes, arranged by George C. Paffenbarger, **Dec. 29.**

VIII. **AAAS Section Np—Pharmacy, American Pharmaceutica Association, et al.** Vice Presidential address of Rudolph H. Blythe, **Dec. 27**; symposia on the Significance of Cosmetics in Medical Practice, cosponsored by the Committee on Cosmetics of the American Medical Association, and on Tablet Manufacture, arranged by John E. Christian, **Dec. 27, 28.**

SOCIETAL PROGRAMS

Alpha Epsilon Delta. Symposium: Problems in Premedical Education, and Annual Luncheon and address by Joseph C. Rinsey on Methods of Improving Liaison and Cooperation between Medical and Liberal Arts Colleges, **Dec. 29.**

American Association of Clinical Chemists. Symposium on Clinical Chemistry.

American Association of Hospital Consultants. Symposium.

American Physiological Society. Symposium: Recruitment and Training of Biological Scientists by High Schools and Colleges.

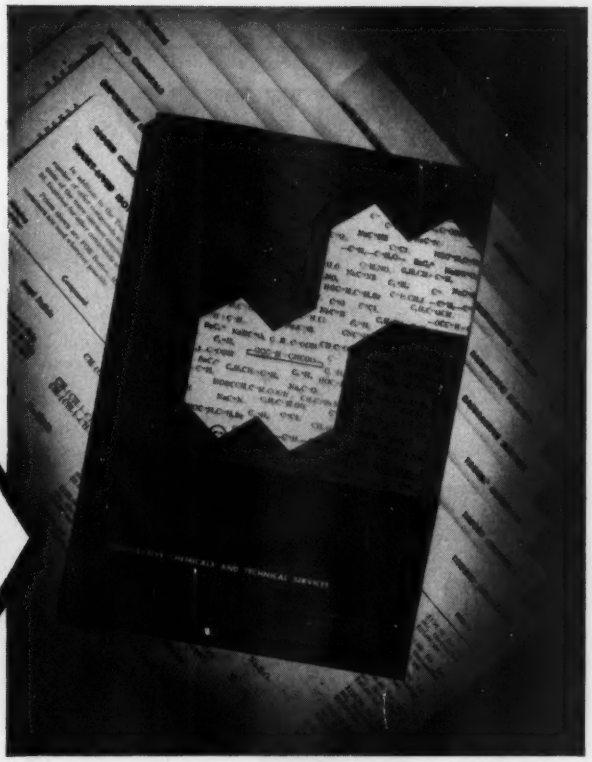
American Psychiatric Association. Two- or three-session symposium: Epidemiology of Mental Disorder, to commemorate 100th anniversary of birth of Emil Kraepelin, with such speakers as Zubin, Kallman, Leighton, Rennie, Clausen, Downing, Gruenberg, Knobloch, and Pasamanick, **Dec. 28, 29.**

American Statistical Association. Symposium: Statistics in Biological Medicine.

History of Science Society. Symposium: The History of Medicine, **Dec. 29.**

The AAAS Annual Exposition of Science and Industry and the AAAS Science Theatre, with recent foreign and domestic films, will be prominent features of the meeting. **Coupons for sleeping accommodations and advance registration will be found in SCIENCE, beginning July 20, and THE SCIENTIFIC MONTHLY, beginning August.**

NEW CATALOG



of radioactive chemicals and technical services

This catalog is the most comprehensive ever published in the nuclear field. It describes the consulting, analytical and research services offered by Tracerlab together with a listing of the isotopically labeled compounds and radiation sources now available. These products are the result of our full decade of experience in the design, development and manufacture of nuclear instruments, as well as our long record of consulting on radiochemical problems.

Prepared specifically for those engaged in nuclear chemistry and applications of radioactivity, it will prove a useful and helpful reference in many ways.

WRITE FOR YOUR FREE COPY TODAY.

CONTENTS

TECHNICAL SERVICES
Consulting
Radioanalytical Services
Training Courses
Procurement of Radioactive Materials

ISOTOPICALLY LABELED COMPOUNDS
Carbon-14 Labeled Compounds
Alphabetical List, C-14 Labeled Compounds
Deuterium Labeled Compounds
Tritium Labeled Compounds
Important Radioisotopes Available for Labeling Chemicals
Short-lived Isotope Labeled Compounds
Industrial Process Tracers
Radioiodine Services

RADIOACTIVE SOURCES
Radiation Reference Sources
Isotope Sources Not Requiring AEC Approval
Radioisotopes for Educational Use
Medical Sources
Radiography Sources
Multicurie Sources for Radiation Chemistry

USEFUL TABLES
Beta Emitters
Hard Gamma Emitters
Available Radioisotopes Listed According to Half-life

Tracerlab

130 High Street, Boston 10, Mass.
2030 Wright Avenue, Richmond 3, Calif.

New York • Houston • Philadelphia • Paris • Cleveland • Los Angeles
Washington • Chicago • Ottawa

Worldwide services in isotopes, instrumentation, consultation and health physics.

NEW!

FISHER PRESENTS

Standard Packaging: Six 5-pt. bottles per ICC approved carton. Meets A.C.S. specifications.

"Dispos-It"* *no deposit*

ACID CASES & BOTTLES

- No Deposit . . . No Paperwork . . . No Breakage Loss
- Compact . . . Lightweight . . . Easier to Handle
- Disposable When Empty . . . Stack Better When Full

Now – Fisher Reagents, as listed at the left, are available in six 5-pint disposable bottles per case. Gone are advance deposits, breakage losses, complicated paperwork for credits. Instead–Fisher Reagents, packaged in the new "Dispos-It," give a convenience, economy and safety factor never offered before. Added features include the "dripless sleeve" fitted on every bottle.

Next time, order reagents in the new Fisher "Dispos-It."

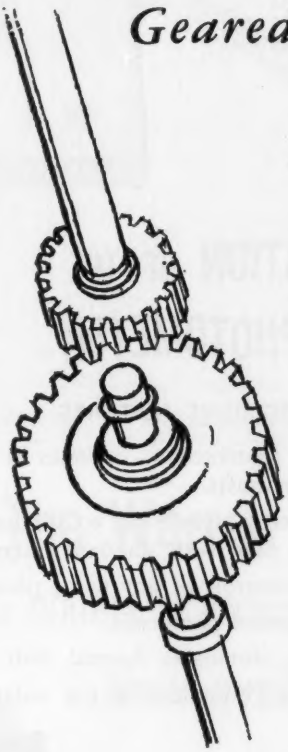
"Dripless Sleeves" too! Unique polethylene sleeve protects hands, keeps handling surface dry, prevents label defacing.

B-3-8 *Copyright 1956

FISHER SCIENTIFIC

Boston Chicago Detroit Philadelphia St. Louis Montreal
Buffalo Cleveland New York Pittsburgh Washington Toronto

America's Largest Manufacturer-Distributor of Laboratory Appliances and Reagent Chemicals



Geared to your needs . . .

For immediate delivery of quality biochemicals at economical prices, NUTRITIONAL BIOCHEMICALS CORPORATION is geared to serve you.

- AMINO ACIDS: PEPTIDES
- "VITAMIN FREE" CASEIN HYDROLYSATE
- NUCLEOPROTEINS, PURINES, PYRIMIDINES
- MISCELLANEOUS BIOCHEMICALS
- VITAMINS
- ENZYMES
- GROWTH FACTORS
- STEROID HORMONES
- BIOLOGICAL SALT MIXTURES
- BIOLOGICAL TEST MATERIALS

NUTRITIONAL BIOCHEMICALS CORPORATION

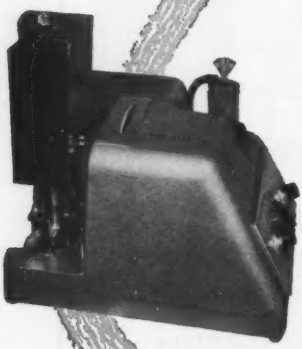
21010 Miles Avenue . . . Cleveland 28, Ohio

Write For
New Catalog
August 1956
Over 1700 Items
Write Dept. 107

XUM

Comparison Proves

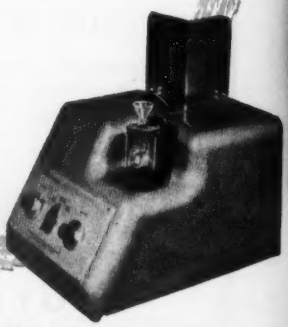
ASK FOR A COMPARATIVE DEMONSTRATION OF THE *Baird* FLAME PHOTOMETER



CHECK THESE EXCLUSIVE FEATURES

- Sealed burner (patented) — operates in smoke, dust or drafts.
- Multilayer filters — results up to 10 times more accurate than with standard filters.
- The simplest controls of any flame photometer.
- Hermetically sealed photocells.
- External flame eliminates thermal drift.
- Smallest Flame Photometer in use today.

The Baird Flame Photometer also provides:
Less washing of glassware . . . double-beam operation (internal standard) for greater inherent accuracy . . . no bracketing . . . no drift . . . greater safety — no bottled gas.



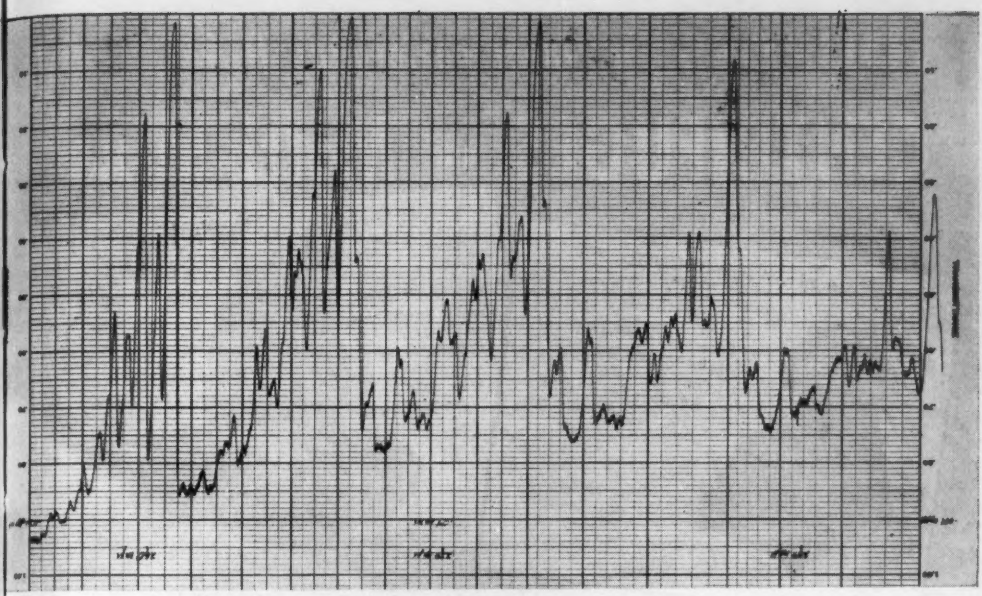
More in use than all other makes combined.

BAIRD ATOMIC

Baird Associates Atomic Instrument Co.
33 University Rd., Cambridge 38, Mass.

Request your live demonstration now.

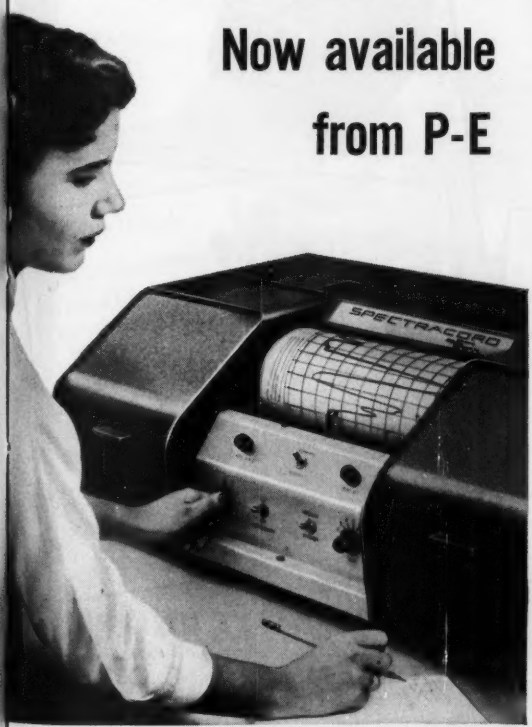
distributed by
ADVANCED INSTRUMENTS, INC.
21 OAKCREST RD. NEEDHAM 92, MASS.



The Warren Spectracord* Unit

th a New DOUBLE Monochromator

Now available from P-E



The Warren Spectracord—well known as a versatile and reliable recording unit for ultraviolet monochromators—has joined the Perkin-Elmer family of products. In addition, a new *double* monochromator, expressly designed for use with the Spectracord is also available from P-E.

A Spectracord equipped with the new double monochromator permits greatly improved performance over the earlier single monochromator combination at a nearly comparable price. Stray radiation is reduced to the vanishing point. Resolution is increased throughout the range. Operation at considerably higher absorbency levels is thus possible.

The Spectracord is still available as an attachment to existing single monochromators. As a Perkin-Elmer product it is now backed by P-E's extensive service and application engineering facilities.

If you are considering expanding your UV facilities, either through additional equipment or by modernizing your present monochromator, why not consider the Spectracord? We will be glad to supply complete information.

*T.M. Warren Electronics, Inc.

Perkin-Elmer
CORPORATION
Norwalk, Connecticut

LOURDES *for finest performing HIGH SPEED*

CENTRIFUGES and Multi-Mixer

Model AA CENTRIFUGE

- Speed to 16,500 RPM
- Force to 34,800 X G
- Capacity — 400 cc
- Adapters for 1, 5, 6, 10, 12 and 15cc tubes

Price: **$515.00** (complete)

Model SL CENTRIFUGE

Swinging Cup Rotor
Speed to 12,000 RPM
Force to 21,500 X G
Capacity — 96 cc

Price: **$565.00** (without rotor)

Volume Rotor
Speed to 11,000 RPM
Force to 20,000 X G
Capacity — 1500 cc

(Complete range of adapters for smaller tubes)

Model AB CENTRIFUGE

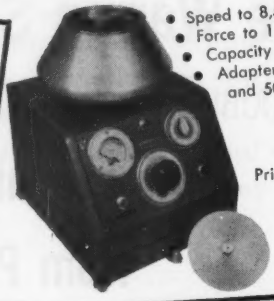
Same performance as model AA above

FEATURES

- 2 hour timer
- Ammeter
- Deluxe construction

Price: **$595.00** (complete)

Model L CENTRIFUGE



- Speed to 8,500 RPM
- Force to 11,800 X G
- Capacity — 1500 cc
- Adapters — 5, 10, 15, and 50 cc tubes.

Price: **$785.00** (complete)

Model AT CENTRIFUGE

Same performance and all features of Models AA and AB above plus . . .

*Centrifugation within a ventilated heavy duty steel tank.

Price: **$745.00** (complete)

MULTI-MIXER

- Homogenizing
- Emulsifying
- Disintegrating
- Fine Chopping

Speed to 16,000 RPM
Sealed mixing within :

- 200cc-SS containers
- 50cc-SS containers
- Quart mason jars
- 15 and 50cc centrifuge test tubes

Price: **$136.00**
Without container or mixing assembly

1 YEAR GUARANTEE ON ALL UNITS!

LOURDES

LOURDES INSTRUMENT CORP.
53rd ST. & 1st AVENUE • BROOKLYN 32, NEW YORK

A DECADE OF SPECIALIZED MANUFACTURING EXPERIENCE

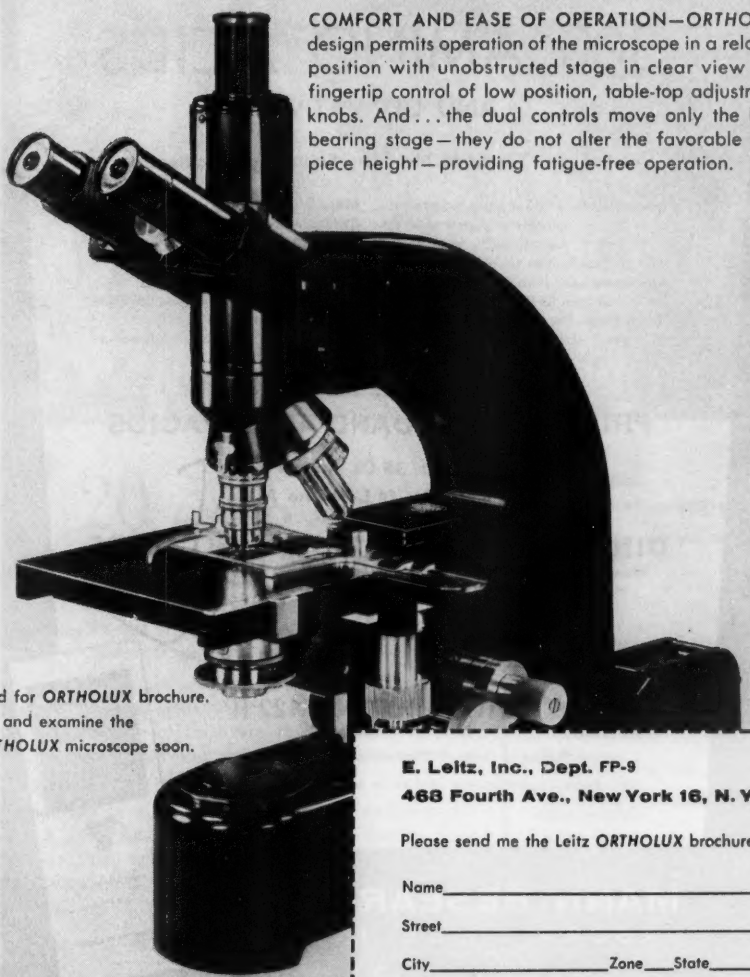
For additional information send for free bulletins **FP-956**

fills every research requirement

Leitz ORTHOLUX RESEARCH MICROSCOPE

IDEAL FOR RESEARCH—The Leitz *ORTHOLUX* microscope is highly valued in leading research laboratories because of its versatility, image quality and unique advantages. The *ORTHOLUX* incorporates the most refined details of advanced optical design and has its own *built-in* illuminating system for transmitted or incident light.

WITHIN SECONDS the *ORTHOLUX* with easily attached Leitz accessories adjusts for: bright and dark field • polarized light • phase contrast • fluorescence microscopy • photomicrography • table and wall projection • ultropak illumination for reflected light.



COMFORT AND EASE OF OPERATION—*ORTHOLUX* design permits operation of the microscope in a relaxed position with unobstructed stage in clear view and fingertip control of low position, table-top adjustment knobs. And . . . the dual controls move only the ball-bearing stage—they do not alter the favorable eye-piece height—providing fatigue-free operation.

Send for *ORTHOLUX* brochure.
See and examine the
ORTHOLUX microscope soon.

E. Leitz, Inc., Dept. FP-9
468 Fourth Ave., New York 16, N. Y.

Please send me the Leitz *ORTHOLUX* brochure.

Name_____

Street_____

City_____ Zone___ State_____

09495

E. LEITZ, INC., 468 FOURTH AVENUE, NEW YORK 16, N. Y.
Distributors of the world-famous products of Ernst Leitz, Wetzlar, Germany
LENSES • CAMERAS • MICROSCOPES • BINOCULARS

1500
MANN ASSAYED
BIOCHEMICALS

300 AMINO ACIDS
AND DERIVATIVES

Purified and Tested to Meet the Following Specifications:

Description—white crystals, crystalline powder or platelets; odorless or nearly so
Ash (sulfated)—less than 0.10%
Ammonia—less than 0.03%
Iron—less than 50 PPM
Phosphate—less than 0.01%
Microbiol—Assay—97—103%
Moisture (free)—less than 0.30%
Chloride—less than 0.02%
Heavy Metals—less than 30 PPM
Sulfate—less than 0.03%
Nitrogen—100 1% on dry basis (accepted literature)
Specific Rotation—100 0.5% (accepted literature)

MANN PHASE SOLUBILITY PRIMARY STANDARD AMINO ACIDS

Kit No. 851: 15 DL Amino Acids
Kit No. 341: 14 L Amino Acids

DINITROPHENYL (DNP) AMINO ACIDS

Kit No. 431: 18 DNP Amino Acids

Literature and information on request

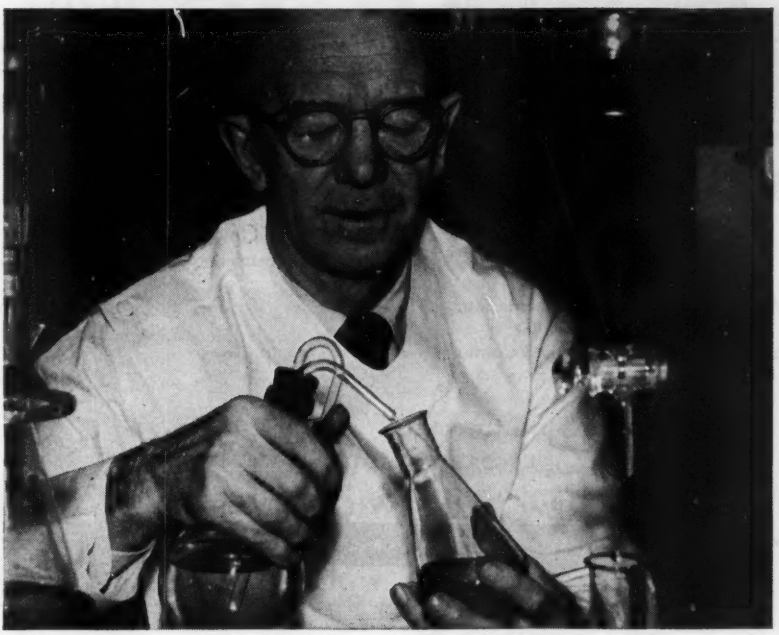
NEW PRICE LIST No. 122 K

A unique catalog of research biochemicals, with detailed specifications used as an accepted reference guide.

MANN RESEARCH
LABORATORIES, INC.
136 Liberty Street • New York 6, N. Y.

research developments

DOW



20 AMINO ACIDS synthesized by Dow

You may have a vital interest in the important developments coming from the Dow laboratories.

Listed at right are seven essential and thirteen nonessential amino acids which have been synthesized by Dow. Three of them are now available in commercial quantities and the rest may be obtained in pilot plant quantities.

Interested in more information about amino acids? Then write us—we will be happy to discuss their potentialities with you. Address THE DOW CHEMICAL COMPANY, Midland, Michigan, Department PH8876-2

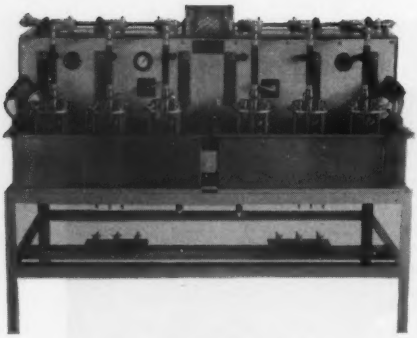
7 essential

L-Isoleucine with D-Alloisoleucine
DL-Leucine
DL-Lysine Monohydrochloride
*DL-Methionine
DL-Phenylalanine
DL-Tryptophan
DL-Valine

13 nonessential

N-Acetyl-DL-Alanine
N-Acetyl-DL-Leucine
*N-Acetyl-DL-Methionine
N-Acetyl-DL-Tryptophan
N-Acetyl-DL-Valine
DL-Alanine
DL-2-Aminobutyric Acid
2-Methylalanine
DL-3-(3, 4-Dihydroxyphenyl)alanine
*Glycine, N.F.
DL-Norvaline
DL-Serine
DL-Tyrosine
*available in commercial quantities.

you can depend on DOW CHEMICALS DOW



An Integrated Pilot Plant
for Aerobic or Anaerobic Fermentations,
Tissue Cultures, and other
Metabolic Studies in Submerged Culture

FERMENTOR DRIVE ASSEMBLY

Model FS600

Accommodates 6 FERMENTORS, stainless steel with Pyrex jar, capacity 5, 7.5, or 14 liters each; readily removed for thorough sterilization in autoclave; special non-freezing, non-bleeding ball bearing agitator housing and seals.

Stainless steel baths, thermostatically controlled within ±½° C., range ambient to 60° C. (approx.); cooling water connections for lower temperatures.

Twin drives for wide range of agitation and aeration rates, speeds 150 to 750 r.p.m.

Complete with flow meters, air filters, metering valves.

UNCONDITIONAL ONE YEAR GUARANTEE

NEW BRUNSWICK SCIENTIFIC CO.

PRECISION · LABORATORY · APPARATUS

P. O. BOX 606 ● NEW BRUNSWICK, N. J.

SERVALL® SS-1

The ORIGINAL Superspeed Angle Centrifuge

It takes pioneering "know-how" to produce operating quality that sets trade standards. That is why leading laboratories and researchers select the SERVALL SS-1, the *ORIGINAL* Superspeed Angle Centrifuge.

CAPACITY: Precision-machined, anodized duraluminum rotor accommodates *eight* 50-ml tubes at a 35° angle in separately numbered compartments. Accessory adapters available for 15-ml, 10-ml, or 5-ml tubes.

ECONOMY: Economical operation is a dividend on low initial cost.

PERFORMANCE: Speeds up to 15,500-rpm exert 31,000 x G. Voltage-adjuster allows continuously-variable speeds.

VERSATILITY: Less than 14″ high—can be operated on a small table, inside refrigerator, deep-freeze, cold room. Rotor easily adapted for use in SERVALL REFRIGERATED CENTRIFUGE.

SAFETY: Dynamic balancing with self-centering, no-thrust drive—Originated by SERVALL—promotes smoothness, long bearing-life, and rapid sedimentation.

Type SS-1A, same as SS-1 but with water-cooled bearing assembly for extra-heavy duty.

SERVALL SS-1 and SS-1A Angle Centrifuges have built, maintained, and are adding to their international reputation as "the workhorses of the modern laboratory."

Write for Bulletin FP-91

Ivan Sorvall, Inc.

P.O. BOX 230,
NORWALK, CONN.

SPENCER

CYCLOPTIC

AMERICA'S NEWEST and FINEST STEREOSCOPIC MICROSCOPE

Outstanding Features

- True three-dimensional erected image
- Top quality optics...low reflection coated
- Reversible and inclined body
- Long working distance
- Large field of view
- Wide range of magnifications
- Broad selection of models
- Dove-gray EPOXY finish
- Advanced styling and design
- LOW PRICE

Plus—

- Revolutionary MAGNI-CHANGER

Desired magnifications simply "dialed-in" by rotation of calibrated cylinder

Manufacturer of the World's Most Modern...Versatile... and Complete Line of Stereoscopic Microscopes

American Optical

INSTRUMENT DIVISION BUFFALO 15, N.Y.

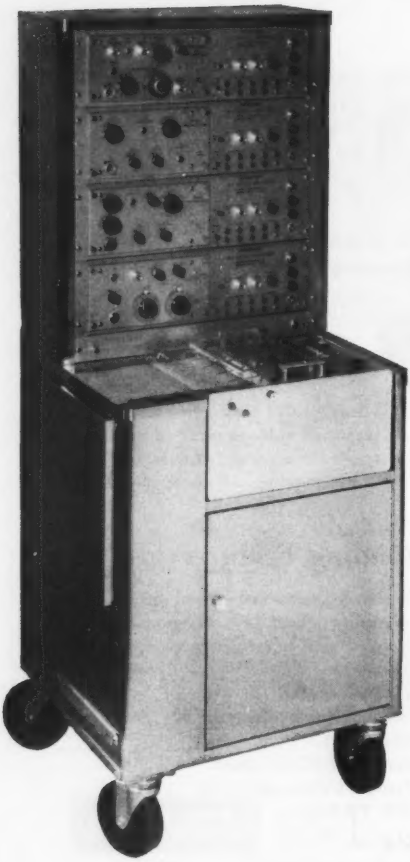
NEW BROCHURE JUST OFF THE PRESS... WRITE FOR YOUR COPY

Dept. 202
Gentlemen.
Rush new CYCLOPTIC brochure, SB56

Name _____

Address _____

City _____ Zone ____ State ____



NEW

GRASS
MODEL 5
POLYGRAPH
FOR
VERSATILE
RECORDING
IN
PHYSIOLOGY
PHARMACOLOGY
ANESTHESIOLOGY
PSYCHOLOGY

- The Polygraph records blood pressure, oxygen tension, CO_2, plethysmogram, respiration, temperature, PGR, EMG with integration, EKG and/or heart rate, EEG and other physiological data.
- Interchangeable Preamplifiers, designed specifically for each application, plug into identical Driver Amplifiers.
- Amplitude of direct-writing oscillographs is 50 mm with 2% linearity.
- Dual channel bench model available for teaching and portability.
- Built to Grass precision standards to insure dependability and accuracy for years of service.

GRASS INSTRUMENT COMPAN

101 OLD COLONY AVENUE - QUINCY, MAS

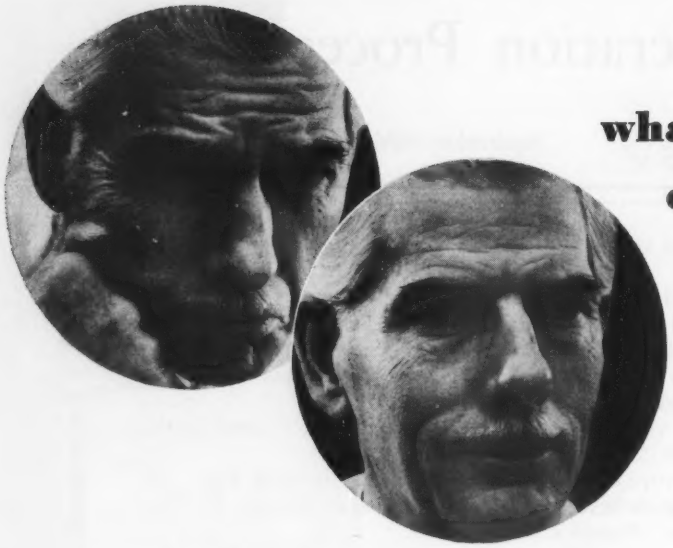
INSTRUMENT MAKERS TO THE MEDICAL PROFESSION SINCE 19

Federation Proceedings

VOLUME 15 *September 1956* NUMBER 3

CONTENTS

NOTES ON THE FORTIETH ANNUAL MEETING OF THE FEDERATION 875
PROBLEMS OF SCIENTIFIC MANPOWER. *Harry P. Smith* 877
NATIONAL REGISTER OF SCIENTIFIC AND TECHNICAL PERSONNEL 880
FEDERATION PLACEMENT SERVICE 881
JOINT SESSION OF THE FEDERATION ON NUTRITION AND CARDIOVASCULAR DISEASE
Nutrition and Atherosclerosis. *L. N. Katz, J. Stamler and R. Pick* 885
Biochemical Aspects of Atherosclerosis. *Christian B. Anfinsen, Jr.* 894
Dietary Aspects. *Fredrick J. Stare* 900
INTER-SOCIETY SESSION ON NUTRITIONAL AND TOXICOLOGICAL STUDIES ON IRRADIATED FOODS
Introductory Remarks. *T. E. Friedemann* 905
Effect of Irradiation Sterilization on Nutritive Value of Protein and Energy of Food. *B. Connor Johnson and V. Chalam Metta* 907
Wholesomeness of a Gamma-Irradiated Diet Fed to Chickens. *C. H. Burns, L. E. Brownell and H. C. Eckstein* 910
Toxicity of Lipid Peroxides in the Rat. *John S. Andrews, James F. Mead and Wendell H. Griffith* 918
Radiation Effects on Water Soluble Vitamins in Raw Beef. *H. D. Alexander, E. J. Day, H. E. Sauberlich and W. D. Salmon* 921
Effect of Ionizing Radiations on Vitamin K. *Luther R. Richardson, Patricia Woodworth and Sandra Coleman* 924
Wholesomeness and Possible Carcinogenicity of Radiated Foods. *L. J. Teply and B. E. Kline* 927
Effects of Irradiation on Wholesomeness of Mixed Organ Meats in Diet of the Rat. *Edward C. Bubl and Joseph S. Butts* 930
Wholesomeness of Gamma-Irradiated Foods Fed to Rats. *H. F. Kraybill, Merrill S. Read and T. E. Friedemann* 933
SYMPOSIUM ON BIOLOGY OF AGING
Aging as a Biological Problem. *N. W. Shock* 938
Age Changes in the Morphology of Tissues and Cells. *Warren Andrew* 942
Cellular Physiology and Aging. *L. V. Heilbrunn* 948
Cellular Metabolism and Aging. *Charles H. Barrows, Jr.* 954
Comparative Physiology of Aging. *A. I. Lansing* 960
SYMPOSIUM ON AMINO ACIDS AND PROTEINS
Amino Acid Imbalance. *C. A. Elvehjem* 965
Metabolism of Glycine: The Succinate-Glycine Cycle. *David Shemin* 971
Characteristics of Kwashiorkor (Sindrome Pluricarencial de la Infancia). *Nevin S. Scrimshaw, Moisés Behar, Guillermo Arroyave, Fernando Viteri and Carlos Tejada* 977
NEW MEMBERS ELECTED, SPRING 1956 986
CORRECTIONS AND ADDITIONS TO MARCH ISSUE 991



what a difference 64,000 lumens make!

difference between viewing of a steady brilliant image or eye-straining squint at a dim flickering one.

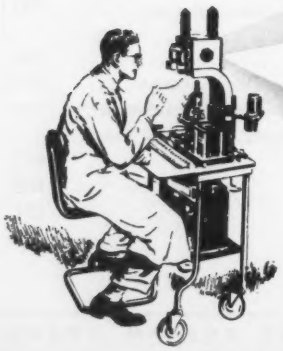
difference between projecting bright images up to ten feet across under the highest powers of the microscope or enduring low magnifications that do not show sufficient detail for a definitive diagnosis.

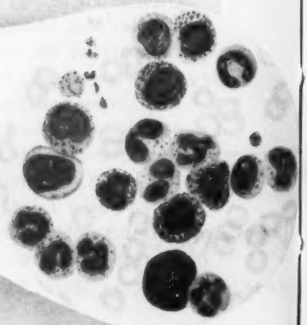
difference between the blue-white color of Scopicon projection which displays biologic stains to best advantage or varying color temperatures which distort color values.

difference between a steady light which does not require further adjustment or a flickering, wavering light source requiring constant recentering.

Scopicon®

ULTIMATE IN MICROPROJECTION





SCOPICON, INC.
Chauncey, New York
Paris Office: COMPAGNIE TECHNICON
7 rue Georges Ville • Paris

NOTES ON THE FORTIETH ANNUAL MEETING OF THE FEDERATION

Atlantic City, April 16–20, 1956

THE FEDERATION MEETINGS in Atlantic City this year were the largest in the history of the Federation; 1915 papers and 6 motion pictures were presented in 186 scientific sessions, with an additional 162 papers listed in the program as read by title. Total registered attendance was 7380, including 6476 members and non-member scientists, 435 guests, 434 exhibitors and 35 press representatives. Approximately 150 exhibit booths were occupied with displays by 102 industrial firms, 10 institutions and 21 members.

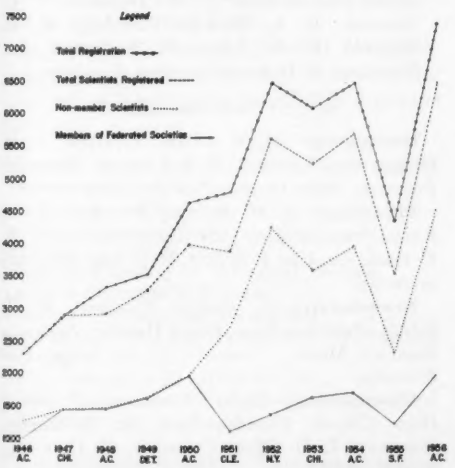


FIG. 1. Registered attendance at Federation Meeting for the period 1946–1956. A.C.—Atlantic City; CHI.—Chicago; DET.—Detroit; CLE.—Cleveland; N.Y.—New York City; S.F.—San Francisco.

Figures 1 and 2 show growth of the Federation Meetings from 1946 to 1956 in terms of attendance and communications presented. During this 10-year period, which followed a suspension of three meetings during World War II, all of the present Member Societies had programs. The American Association of Immunologists, which joined the Federation in 1942, held joint sessions with the American Society for Experimental Pathology at earlier meetings but presented its own program at the 1946 meeting. The exhibits section became a regular part of the Annual Meetings in 1948, increasing total registration by 350–450 since that time. Guests of members and non-member scientists and press representatives make up the balance included in the total registered attendance.

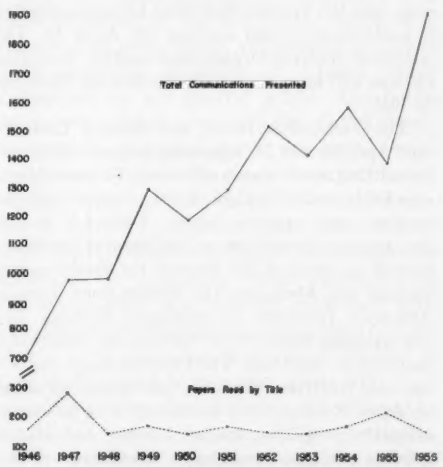


FIG. 2. Communications on the programs of Federation Meetings from 1946–56. Communications presented include papers on joint sessions, symposia and panel discussions, motion pictures and demonstrations.

The American Institute of Nutrition, host society for the 1956 meeting, sponsored the Joint Session. With Dr. George R. Cowgill presiding, Drs. L. N. Katz, C. B. Anfinsen, Jr., and F. J. Stare presented the clinical, biochemical and dietary aspects of Nutrition and Cardiovascular Disease. These papers appear in this issue of FEDERATION PROCEEDINGS.

This year marks the Fiftieth Anniversary of the American Society of Biological Chemists. Progress and change in chemical concepts, teaching practices at the undergraduate and graduate levels, and the history of the Society and *The Journal of Biological Chemistry* were reviewed in two special symposia and at the 50th Anniversary Banquet.

Papers from the symposia and remarks of the speakers at the banquet appeared in the July issue of FEDERATION PROCEEDINGS.

Seventeen Intersociety Sessions, (3 on atherosclerosis, 3 on blood clotting, 2 on cholinesterase, 4 on tumors and 5 on radiation biology) and an Intersociety Symposium on Irradiated Foods were held. Five additional society symposia presented during the week included two in the Physiology Program on the Biology of Aging and Teaching Physiology in Undergraduate Schools; a Nutrition symposium on Proteins and Amino Acids; an Immunology symposium on Antibody Formation; and a joint symposium on Synergism by the Pharmacology and Biometrics Societies.

The American Society for Artificial Internal Organs and the Histochemical Society held scientific sessions and business meetings April 15 and 16 in conjunction with the Federation Meetings, and the Poultry Nutrition Group conducted a conference in two sessions on April 15. The Artificial Internal Organs and Poultry Nutrition Groups will meet during the Federation Meetings in 1957.

The Federation Board and Society Councils met April 15 and 16, reporting actions taken and submitting new business at Society Business Meetings held April 17 and 19. A list of officers for the coming year appears below. Editorial boards and special committees of the Member Societies, as well as those of the Society for Experimental Biology and Medicine, the Walter Reed Society, American Institute of Biological Sciences and the National Institutes of Health used Federation facilities for meetings. The Pharmacology, Pathology and Nutrition Societies held dinner meetings on April 18 followed by presentation of awards or scientific programs; special interest and alumni groups met for breakfasts, luncheons, dinner meetings and social gatherings throughout the week.

A Ladies Committee, headed by Mrs. Karl Habel, provided a hospitality desk in the registration area and conducted an interesting program of activities for wives of member and non-member scientists.

The Public Information Committee, headed by Dr. J. O. Hutchens, sponsored two educational programs in Philadelphia, April 20. High school science teachers and student counselors in the Philadelphia area were invited to a Career Forum in Biology. Drs. J. M. Luck, H. C. Hopps and M. W. Chase spoke on the topics of professional opportunities, specialization and preparation for specialization in biology. The speakers were introduced by Dr. Hutchens who presided over a question-and-answer period following the discussion. The staff and graduate students at the University of Pennsylvania Hospital and Medical Schools were hosts to college students in biology at an open house in the University's research and teaching laboratories. The tours and demonstrations were followed by a buffet supper and panel discussion. Dr. J. H. Comroe, chairman of the panel, introduced Drs. C. B. Anfinsen, Jr., J. L. Orbison and John Field II who discussed professional opportunities for biologists, preparation for specialization, and how biologists work and think. Dr. Comroe led a question-and-answer period following the discussion. The Federation is indebted to the University of Pennsylvania for providing facilities for these meetings and to Smith, Kline and French Research Foundation for the buffet supper.

MEMBERS OF THE FEDERATION BOARD 1956–57

Jules Freund, *Chairman*, M. W. Chase, F. S. Cheever, *Immunology*; A. C. Burton, L. N. Katz, W. F. Hamilton, *Physiology*; H. E. Carter, Philip Handler, J. M. Luck, *Biochemistry*; Harry Beckman, H. C. Hodge, E. G. Gross, *Pharmacology*; F. W. Hartman, C. C. Erickson, H. L. Stewart, *Pathology*; R. R. Williams, R. W. Engel, W. H. Sebrell, Jr., *Nutrition*; M. O. Lee, *Federation Secretary*.

SOCIETY OFFICERS, 1956–57

Immunology—M. W. Chase, *President*, J. H. Dingle, *Vice-President*, F. S. Cheever, *Secretary-Treasurer*, Jules Freund, *Past President*.

Physiology—A. C. Burton, *President*, L. N. Katz, *President-Elect (Secretary-Treasurer)*, W. F. Hamilton, *Past President*, M. O. Lee, *Executive Secretary*.

Biochemistry—E. Carter, *President*, J. T. Edsall, *President-Elect*, Philip Handler, *Secretary*, Stanford Moore, *Treasurer*, J. M. Luck, *Past President*.

Pharmacology—Harry Beckman, *President*, Otto Krayer, *President-Elect*, H. C. Hodge, *Secretary*, J. M. Dille, *Treasurer*, E. G. Gross, *Past President*.

Pathology—F. W. Hartman, *President*, E. D. Warner, *Vice-President*, C. C. Erickson, *Secretary-Treasurer*, H. L. Stewart, *Past President*.

Nutrition—R. R. Williams, *Acting President (Vice-President)*, R. W. Engel, *Secretary*, J. B. Brown, *Treasurer*, W. H. Sebrell, Jr., *Past President*.

THE 1957 ANNUAL MEETING

The next annual meeting of the Federation will be in Chicago, Illinois, April 14–20, 1957. Headquarters will be at the Conrad Hilton and Palmer House, and scientific sessions and other meetings will be held at these and the Sheraton-Blackstone and Congress Hotels.

PROBLEMS OF SCIENTIFIC MANPOWER[1]

HARRY P. SMITH

SEVERAL YEARS AGO the Federation cooperated with several other professional and scientific societies in organizing the Scientific Manpower Commission. Its role is to study the problems involved in securing and maintaining scientific and technological manpower adequate to meet the needs of this country. When the Commission was first organized, the Federation was represented by Doctors Milton O. Lee and Joseph H. Roe. Doctor H. P. Smith succeeded Doctor Roe on January 1, 1955. Since three years have now elapsed since the Commission was organized, the two current representatives of the Federation conferred and decided that enough data had accumulated to justify the making of a progress report to the Federation Board.

As now constituted, the Scientific Manpower Commission accepts nominations to membership from each of the following ten scientific organizations:

American Association for the Advancement of Science
American Astronomical Society
American Chemical Society
American Geological Institute
American Institute of Biological Sciences
American Institute of Physics
American Psychological Association
Federation of American Societies for Experimental Biology
Policy Committee for Mathematics
Policy Committee for Scientific Agricultural Societies

There are 20 members of the Commission; Doctor Howard A. Meyerhoff is the Executive Director. The offices are at 1507 M Street, N.W., Washington 5, D. C.

The Commission is faced with complex problems involving personnel needed by pure science of abstract nature at one end of the spectrum, and for the technological needs of industry at the other. These diverse problems muse be analyzed together, since the educational policies of schools, colleges and universities must be geared to train men and women over this entire range.

In approaching these problems, the Commission at once became interested in ascertaining the present size of the scientific manpower pool, and the magnitude of the demand for increasing it. In a very real sense, the need for basic research and for education in fundamentals is limitless. Achievements are limited only by the availability of talented students and scientists, and by the willingness of the public to support their activities.

The need for applied science is related a little more intimately to the day-by-day demands of industry and government, and is more subject to being measured. Moreover, it is more readily understood by the general public. The public listens with interest when told that Soviet Russia is currently producing 60,000 well-trained engineers a year, whereas the American output is only one-half as great. Early in its career, the Scientific Manpower Commission made a special study of the needs of American industry with respect to scientists. It concluded that approximately 120,000 scientists are currently employed, aside from the professionally trained engineers who are known to be somewhat in excess of 400,000. It may be of interest to note that about 50% of all scientists employed by industry are classified as chemists, 9% are physicists, 7% are geologists, 6% are mathematicians and biologists, and 2% are psychologists. Employment statistics showed that scientists in industry have been increasing in numbers recently at a rate of about 15% a year. More impressive still, as a gauge of demand, is the aggressive activity of industrial concerns in recruiting recent graduates in science and engineering. The demand is obviously in excess of the supply.

The need for teachers from elementary school to university is also of interest and is determined in part by the varying levels of student enrollment; this, in turn, is dependent in part upon birth rates. It is common knowledge that birth rates were low during the depression years. It is not correct to say, however, that the population declined during the depression. One should say instead that it did not increase as rapidly as in earlier or in later years.

[1] Report submitted to the Federation Board at Atlantic City, April 15, 1956.

The relative stagnation in population during 1931–41, and the rapid rise subsequently, initiated a wave-like shift in enrollment in the public schools, colleges and universities. This, too, is widely known. However, the exact timetable is not appreciated by all. From data supplied by the Bureau of the Census one can show that the period of stagnation began in the elementary schools as early as 1937; it grew steadily worse for ten years. There followed a decade of rapidly increasing enrollment in the elementary schools. That oncoming wave is now tapering off into a slow, steady increase.

High school enrollment has been passing through the period of stagnation during the past ten years. The crest of the rising wave is now approaching the high schools; the rise can be expected to continue for an additional decade. The colleges, on the other hand, should have entered the period of stagnation in about 1949. Actually, however, military service in World War II brought on the stagnation at an earlier date. Moreover, the discharge of veterans in 1945 and 1946 provided an artificial surplus of older students for the next few years. The Korean war also created irregularities which were superimposed on the wave-like progression associated with the earlier changes in birth rates. The long-range effect of the depression still continues, however. The final effects on college enrollment will persist until about 1970. In the meantime the colleges are ill prepared to supply the large number of teachers needed in the elementary schools and high schools.

The societies which constitute the Federation are of course particularly concerned with education at the graduate level. It is common knowledge that the supply of well-qualified graduate students has fallen off in the past three years. Economic factors have been blamed, and obviously they are important. However, the scarcity of graduate students is also related to the fact that most of the World War II veterans completed both their undergraduate and graduate studies about three years ago. The current supply of graduate students now comes almost entirely from the group born in the years of the depression—a group which was numerically at least 50% below normal. Unfortunately, we will continue for another seven years to recruit our graduate students from that under-sized group. Even when the wave of recovery does reach the graduate school, it will climb slowly for approximately a decade. Not until 1975 will the graduate colleges escape altogether from the aftermath of the depression. These are facts which should be emphasized. A decline in the number of qualified graduate students could have a serious effect upon the future supply of scientists needed by universities, colleges, industry and government. The situation is all the more alarming in view of the growing demand by all of them.

It is obvious that nothing can be done about the size of the age group which originated during the depression. The only hope lies in making maximal use of the talent which does exist in that age group. It has been shown, for example, that ordinarily only about 50% of the really talented students of our high schools enter college. It is agreed that every effort should be made in the years which lie ahead to avoid this waste of talent. Likewise, the graduate colleges may succeed in recruiting a somewhat larger percentage of students who ordinarily stop with the baccalaureate degree.

The problem of recruiting students for undergraduate colleges and for the graduate schools is partly economic and can be solved to some extent by liberal use of scholarships. However, there is also a factor of faulty motivation of high school and college students, particularly with respect to science. The 'flight from science' is to a considerable degree the fault of the high schools, for it is there that the students receive their first real opportunity to become acquainted with science. Unfortunately, science is receiving far less attention in high schools than was the case several decades ago. It is reported, for example, that only 20% of recent high school graduates have had courses in physics, whereas the percentage was three times that high 50 years ago. High school training in chemistry and mathematics is also being neglected. Students who enter college thus lack motivation for science. Even those who do elect science courses in college tend to be handicapped by the deficiencies in the training which they received in high school. The most distressing thing of all is that the situation in high school will probably grow worse for a decade or so, because the enrollment is rising and the shortage of competent teachers is becoming more acute. These problems have been faced squarely in a number of symposia which have been held in recent years.

One encouraging thing can be noted—the fact that the Scientific Manpower Commission is not

the only organization which is devoting its energies to these problems. The Commission welcomes the support of these other groups. It collaborates closely with the Engineering Manpower Commission. The two organizations have joined hands in fund-raising and in the publication of a newsletter. Both are in close contact with the Future Scientists of America Foundation, which was established by the National Science Teachers Association. The two Commissions also maintain close contact with the National Science Foundation, the National Research Council and the Office of Defense Mobilization. Only recently, President Eisenhower appointed a National Committee for the Development of Scientists and Engineers. It is to be hoped that this organization will extend and help consolidate the continuing achievements of other private and governmental agencies.

Among the things to be done, the Scientific and Engineering Manpower Commissions have recognized first of all the very great urgency of strengthening the high schools of the entire country. They have received extensive cooperation of industry at the local level in doing this. Industry has cooperated in stimulating the interest of high school students in science and has assisted various organizations in their efforts to procure science teachers in large members. To a considerable extent, this is a problem of public relations, involving the general public, and the supervisors of our public schools as well as the high school teachers and students.

Finally, the Scientific and Engineering Manpower Commissions have sought to minimize the wastage of scientific and engineering talent of the country. They have been instrumental in securing military deferment for teachers and for science students in colleges and universities. Their officers have testified before congressional committees and have thus been instrumental in helping to formulate the Reserve Forces Act of 1955. This Act makes provision for an abbreviated training period of three to six months, in lieu of a full two-year period of active duty. The candidate who selects the shorter period is obligated for a longer period of training in the Active Reserve. The Act makes provision, moreover, for continuous screening of the Ready Reserve, with transfer to the Standby Reserve of individuals possessed of certain 'critical' skills. These provisions tend to reduce the wastage of civilian manpower in such critical fields as science and engineering.

In conclusion, it is suggested that we in the Federation can well afford to review our policies of graduate education, in light of current problems of recruitment. Our problem seems to be partly one of competing with other disciplines for our share of able students. It is also in part a matter of making maximal utilization of what we have. It is a fair question as to how far we should go in directing the energies of our young scientists into the research of clinical departments, for these researches center mainly about the various categories of disease. The recent report of the C. N. H. Long Committee pointed clearly to the dangers involved in expanding categorical research at the present time. This Committee advocated increasing emphasis on basic research. This advice is consistent with the policy of the National Science Foundation in stressing fundamental research. We might well ask ourselves whether we are doing all we can to retain men of promise in our own departments. Your representatives to the Scientific Manpower Commission will welcome any suggestions the Federation has to offer, both in the formulation of policy and in presenting policy decisions to our colleagues on the Commission.

NATIONAL REGISTER OF SCIENTIFIC AND TECHNICAL PERSONNEL

THE National Register of Scientific and Technical Personnel is an undertaking of the National Science Foundation and its cooperating agencies. The Federation of American Societies for Experimental Biology and the American Institute of Biological Sciences are the two organizations that are preparing and maintaining the Register of Biologists. In preparing the 1956 questionnaire and check list, the Federation had the advice and guidance of several members of the constituent Societies of the Federation. Their suggestions and ideas were incorporated in the new format, expanding the fields and disciplines in experimental biology, with the result that scientific skills can now be more accurately classified.

A 'Central Record Office' has been established in Raleigh, North Carolina, by the NSF. Its function will be that of a central clearinghouse for information concerning all scientific and technical personnel in the United States.

Practical use has been made of the Register within the past year. The Public Health Service requested and was supplied with a list of Russian speaking scientists in the field of experimental biology. The Register cooperated in a national cancer survey and has been active in Federal Civil Defense exercises simulating disaster. It is our hope that the facilities of the Register will be used with greater effectiveness as the year progresses.

The Society of American Bacteriologists has requested that it be included in the 1956 roster. Their membership is close to 5,000 and it will be of decided advantage to include them in the Register.

The National Register of Scientific and Technical Personnel has now included in its files most of the biologists in the United States who are members of one or more of the biological societies. It is desired, however, to go beyond Society membership and to identify and include all qualified professional biologists. An important group among those not yet included are many biologists teaching or doing research in the liberal arts colleges. In order to reach this particular group, letters have been sent recently to Heads of Biology Departments of these colleges, requesting names and vitae on all biologists.

STATISTICS ON FEDERATION REGISTER (MAY 1-JULY 18, 1956)

Questionnaires mailed—13,433

	Respondents	Non-Respondents
Members	2,484	1,850
Non-Members	2,613	1,486
SAB	2,282	2,718
Total	7,379	6,054

Previous to the 1956 mailing of 13,433 questionnaires in May, the Federation Register checked its files against the central strip list of the eight collecting agencies, in order to avoid unnecessary duplication. To reduce this hazard further, space has been provided at the bottom of the cover letter, whereby information can be recorded for the individual's personal file. The Federation Register office is fully cognizant of the 'nuisance value' of duplication, and any second questionnaire received from this office will be in the nature of a re-mailing to non-respondents. This re-mailing of the 1956 questionnaires will be in late October or November.

RUTH C. HABEL
Federation Register

FEDERATION PLACEMENT SERVICE

With the current interest in increased demand for scientists of all kinds, and with the large increase in funds from governmental and other sources granted to academic and research institutions in the biological field, a reflection of these factors could be expected in the over-all activities of the Placement Service of the Federation of American Societies for Experimental Biology. Therefore a report on these activities may be of general interest.

During the past six years the Placement Service has continued along much the same course, serving as a clearing house of information between employers and candidates, modifying its procedures only in minor adjustments to increase efficiency and effectiveness. There were, however, two major changes in 1951. For about three years before that time lists of candidates had been prepared semiannually with monthly corrections and supplements. In 1951 these were changed to quarterly lists with no supplements and at the same time semiannual lists of positions and an annual list of fellowships were initiated. The second major change came with the decision of the Executive Committee of the Federation in April 1951 to establish a subscription price for employers who wished to receive the lists and to utilize the interview service at the annual meeting. This has since been increased and an annual subscription is now $15.00. Single copies can also be obtained. Simultaneously the annual charge to candidates for maintaining registration was raised from $1.00 to $3.00. These increases have had no apparent adverse effect on the numbers of candidates or of employers who use the Service. The Federation continues to bear about half the direct cost of operation of the Service. This seems desirable, keeping the activities wholly under the policies and supervision of the professional group and in no way competing with commercial agencies.

In the first years, during which the Placement Service came under the direction of the newly-established central office of the Federation, when regular lists of candidates were prepared and when it first was assigned to the undivided attention of one person, there was a marked yearly increase in the numbers of employers and applicants using the Service. This increase has now leveled off. This might indicate it is meeting the present needs of employers in the field within the limitations of the kind of service possible with the present staff. As mentioned before, the fees required have not seemed to affect the number of users, but the location of the annual meeting definitely has. There was a considerable drop in applicants in 1955 when the meeting was in San Francisco from the high of 1953 when it was held in Chicago. This was anticipated, as not many graduate students or recent Ph.D.'s from the eastern part of the country, where graduate centers are numerous, can afford a trip of that distance.

It has become evident that there is a definite advantage to candidates in registering well in advance of the annual meeting in order to have their names included in the February list if they plan to use the interview facilities there. This gives employers time to select candidates whom they wish to interview in advance of the hurried days of the meeting, and to correspond with them and their references. As this fact and the general routine procedures of the Service have become better known, advance registration of candidates for the meetings has increased and the effects of the location of the annual meeting begin to show in the registration several months before the actual meeting dates. Hence, the most significant yearly totals are prepared on a calendar year basis rather than from March 1 to March 1 as had been done in earlier years.

In table 1 the location of the meeting date is given for the ensuing meeting in April. Thus the caption '1953–54 Atlantic City' means the meeting of April 1954 was in Atlantic City. Advance registration for this meeting shows in the total registration of 792 for that year, but this large total also includes the registration at Chicago the previous April. While these figures show the yearly totals for candidates they do not indicate the effect of the meeting place. In addition, candidates who register in advance were counted in both years, being on the rolls before March 1 and often not becoming inactive until later in the summer, so were counted again in the next March 1–March 1 totals. This accounts for the higher number in the March 1–March 1 tally compared to the one based on a calendar year. From table 2 it will be noted there is a decided seasonal variation in the number of candidates

TABLE 1. CANDIDATE REGISTRATION MARCH 1–MARCH 1

	Physiology	Biochemistry Nutrition	Pharmacology	Pathology	Immunology	Total
1948–49 Detroit	36	48	12	1	7	104
1949–50 Atlantic City	88	137	25	2	3	255
1950–51 Cleveland	173	224	38	7	10	452
1951–52 New York	184	286	43	4	19	536
1952–53 Chicago	259	310	74	12	40	695
1953–54 Atlantic City	263	376	81	9	63	792
1954–55 San Francisco	247	358	71	10	55	741
1955–56 Atlantic City	198	284	54	5	36	577

TABLE 2

	1952	1953	1954	1955	1956
	Candidates on List				
Feb.	171	225	277	189	210
May	299	359	327	223	317
Aug.	202	228	179	151	
Nov.	159	187	142	124	
	Positions on List				
March	92	87	110	130	191
Sept.	94	72	90	101	

TABLE 3. SUBSCRIPTIONS AND CANDIDATE REGISTRATION

	Employer Subs.				Candidate Registration Jan. 1–Jan. 1
	As of March 1	As of May 1	Total Annual	Feb. Single Copy	
1952	217	248	265	37	473
1953	204	231	242	41	525
1954	220	250	265	37	481
1955	198	227	240	11	348
1956	203	234		35	

on the lists, with a low in November when few people are changing jobs and a high in May when many remain on the rolls who registered at the meeting. The August list is again smaller, for most academic appointments have been made by that time and the majority of candidates have made commitments for the year. Thus the total candidate registration in table 3 from January 1 to January 1 is a more accurate indication of individuals available, showing only new registration for that period, with little or no duplication, unless the candidate has registered for a second year.

Table 1 also shows the distribution of the candidates among the fields of the member Societies, with the proportion remaining about the same from year to year. Biochemistry and nutrition have always been listed together; very few record nutrition as their primary field and those who do always have considerable training in biochemistry. Together they have always accounted for about half our candidates.

Employers' annual subscriptions to the lists of the Service are for a calendar year and they are the only actual figures which can be used to assess employer interest and use (table 3). There is no way of ascertaining the actual number of positions which employers may be seeking to fill. While some register a number of openings, others recruit from the lists for positions never registered with the Service. It is not required that employers register a position to subscribe to the lists of candidates or to use the interview facilities at the annual meeting. Hence the number of positions registered with us in any one year can only be considered an approximation of the minimum number of positions the employers have to fill.

The distribution and type of these positions are also indicated by a study of the source of these subscriptions. In table 4 the subscribing groups are classified as university, governmental, research institute, industrial and personal. The university category is further separated into those in medical schools and those in other divisions of the universities. It will be seen that 68% of the subscribers are academic, and 55% of these in medical schools. In addition, some laboratories which are entirely government supported and are categorized as governmental, have university affiliations, often are located on university property and the staff have university appointments. Usually these laboratories are associated with medical schools and are definitely oriented to a medical program. There were two exceptions, these being two governmental laboratories sponsored by the Department of Agriculture. Likewise several of the research institutes have medical connections and it can be assumed that many of the industrial groups which are chiefly in pharmaceutical companies are also primarily interested in medical problems. Hence it becomes evident that while a majority of positions are in the academic field, a still larger number are directly in and limited to the medical field rather than in the wider field of experimental biology.

Any total of positions filled by the Service

TABLE 4. 1956 SUBSCRIBERS

	No.		%	
University:				
Medical schools	134		55	
Other departments	31	165	13	68
Governmental		22		9
Research institute		12		5
Industrial		37		15
Personal		7		3
Total		243		100

prepared from our records would be far from accurate. Both employers and candidates are asked to notify this office when a position is filled following contacts made through the Service. Many do this, but cooperation is far from complete. Not infrequently a candidate writes us he has taken a position procured without Placement Service assistance, when we have in our files a letter from the employer saying he obtained the candidate's name from the lists. It is assumed this occurs because the employer does not tell the candidate of the source of his information. In recent months the employer's obligation to so inform the candidate has been repeatedly stressed in letters and notices with encouraging results. In the few months since the 1956 annual meeting we have had more notices than previously, from both employers and candidates, that positions were filled with help from the Service. It is doubtful that the Service has become more efficient, rather that employers are more careful to inform candidates of their use of Placement Service information.

Since procedures and registration have been comparatively unchanged the past few years, it may be of interest to study these totals in relation to the trend of supply and demand they present. Obviously the candidates represent well-trained scientists, since each time a breakdown of the registrants has been made, all but a small minority had obtained a doctorate. Thus they cannot be confused with those highly trained and skilled technicians, so necessary to present-day industrial and scientific progress, but who, for the most part, are not scientists in the older and more limited connotation of the word. The candidates on the Placement lists have had the training to prepare them to join the search for new scientific knowledge, and most of them are eager to obtain positions offering this opportunity. Likewise most are reluctant to accept posts, even though the salary is attractive, where they will be research associates in some sponsored project, but where they quite clearly will be carrying out directions of someone little

TABLE 5. INTERVIEW SERVICE SUMMARY

Year	Employers	Candidates	% Received Interviews	Total No. Interviews
1951	161	209	74	532
1952	133	332	68	577
1953	155	358	68	597
1954	176	344	64	654
1955	116	165	77	406
1956	205	322	83	1,131

TABLE 6. CANDIDATE REGISTRATION

	Jan.	Feb.	March	At Convention
1953				358
1954	105	49	74	344
1955	64	27	25	165
1956	74	56	68	322

interested in an experimental approach to a problem.

The grant-sponsored research project presents another problem to the young candidate for a scientific job. Most of these projects have a definite time limit, often on a year-to-year basis and dependent upon the interest and available funds of the granting agency. It can be assumed that excellent research work usually receives merited continued support; but a year is a short time to produce definitive results in many fields and even though a young man may be doing acceptable work, the junior member of a team knows his services will be the first to be dispensed with if there is a decrease of funds for any reason whatever. Nor do these project associates usually have an opportunity for building up any future security in a retirement plan as is possible under academic and civil service appointments. Generally candidates have family responsibilities; often they have done some years of military service and feel they can no longer commit their future to work, no matter how interesting, which does not offer reasonable security. With the increase in widely given grants which usually provide for one or more research associates there is an increase in such positions. It would be of interest to know how much they are responsible for the large increase in candidate interviews at the 1956 annual meeting at Atlantic City (table 5). Such grants are still increasing and if they are responsible for this large number of interviews we can expect a still greater number in 1957.

It has seemed to this office that in 1953 a crest was reached in the supply of candidates for the jobs available in the fields covered by the Placement Service. Our candidate registration was highest that year and our number of employer subscriptions was a little lower (table 3). In 1954 we had more employer subscriptions and about

8% fewer candidates. Last year, 1955, there was a drop in both candidates and subscriptions. This was expected with the annual meeting in San Francisco, but it is possible that this was part of the trend noted the year before, since the decrease in candidate registration was greater than the one in employer subscriptions. Or perhaps this only meant it was possible for more employers to travel to San Francisco. In 1956 the employer subscriptions are again a little lower than in 1954, a year of high candidate registration, but the candidate registration is also slightly less than in 1954, at least in the three months preceding the convention held at Atlantic City (table 6). The convention was held at Atlantic City both in 1954 and in 1956, removing the variable factor of location in comparison of the totals.

Another basis of comparison is the number of candidates and positions in the lists for the different years (table 2). The number of candidates on the February list was highest in 1954. That year we had increased early publicity on the advantage of a candidate having his name appear on this list if he planned to attend the annual meeting, with a result of heavier advance registration of candidates planning to use the interview service. By the time of the 1954 May list this total of candidates on the roster had decreased until it was below the one of the previous year in May. As indicated above, 1955 registration was low, but it will be noted the positions on both the semiannual lists of positions had continued to increase. This year we have reached an all time high with 191 positions on the March list of positions. These were all current openings since none were entered unless they had been registered after January 1 or we had received notice that they were not yet filled. It might be mentioned, however, that about half this increase of 1956 was covered by two large blocks of positions, one registered by a government installation, the other by a large pharmaceutical company. Candidates on the roster were somewhat fewer than in 1953 (table 2), so perhaps this proportional decrease in candidates is not a very significant one at present. Substantiating this would be the fact that 81 single copies of the March list of positions were ordered, 20% more than last year.

The interview facilities provided at Atlantic City were exceptionally busy this year (1956). This was not anticipated since advance registration of candidates and of employers who indicated they would be using the interview service was a little lower than in 1954, when we were at the same location. The final registration of candidates (table 5) was in accord with this, as there were 344 in 1954 and 322 in 1956. But we had about 10% more employers in 1956 than two years earlier. More employers who had not been previous subscribers and had never used the service made their initial contact at our Atlantic City office than had done so at earlier sessions. Thus, the figures cited would seem to show that jobs are more plentiful than previously in the groups using the Service as they are in the scientific field in general. While the annual subscription list of employers has changed little in five years, (table 3) a steadily growing number of employers utilize the annual interview service (table 5). The number of candidates has slightly, but steadily, declined since 1953. The greatest change is noted in the number of interviews, with twice as many in 1956 as in any previous year. This had the effect of increasing the number of applicants who received interviews. Until 1955 the percentage of these remained near 70%. In 1955 it rose to 77% due, it has been presumed, to the small candidate registration that year. However in 1956 it rose further to 83%. This would lead one to conclude that employers are having more difficulty in finding candidates who meet their requirements and are interviewing individuals in whom they would not have been interested a few years ago. There is, of course, the possibility that candidate qualifications have become higher or employer requirements lower. No basis for the first can be found in an analysis by objective criteria such as degrees held, or age of candidate, which have remained much the same from year to year. Salary requests have increased on an average of $1500 for recent Ph.D.'s in the past five years, but so have salaries offered. The outspoken reluctance of well-prepared candidates to take the research associate posts mentioned before may contribute to the employer's difficulty in filling them, necessitating more interviews.

This might all be interpreted as showing a steady, healthy growing demand for more workers in the fields of the member Societies of the Federation, which, as was pointed out earlier, is predominately in the medical not general biological field. But the demand scarcely indicates any acutely growing shortage of trained individuals in this somewhat limited division of experimental biology.

LETHA K. ANDERVONT
Assistant to the Director

JOINT SESSION OF THE FEDERATION

Atlantic City, New Jersey, April 17, 1956

Nutrition and Cardiovascular Disease

Chairman: GEORGE R. COWGILL

NUTRITION AND ATHEROSCLEROSIS

L. N. KATZ, J. STAMLER[1] AND R. PICK

From the Cardiovascular Department, Medical Research Institute, Michael Reese Hospital, Chicago, Illinois

NUTRITION is significantly involved in almost all cardiovascular diseases. Its role in atherosclerosis is the focus of attention of this symposium. This is proper because atherosclerosis is by far the most important form of cardiovascular disease. It, together with hypertension, constitutes the greatest cause of morbidity and mortality today. It is estimated that at least 45% of deaths from all causes in this country are the result of atherosclerosis, with or without hypertension. In 1950, so-called arteriosclerotic and degenerative heart disease alone took a toll of over 10,000 deaths in men aged 25–44, and almost 90,000 deaths in men aged 45–64—these being 15% and 36%, respectively, of all deaths in male Americans of these age groups. No wonder atherosclerosis is singled out as the leading reaper of death and disability in this country—the number one killer of middle-aged Americans, as well as the elderly.

This symposium's preoccupation with this problem is further appropriate, since a great deal of new information deserving of review has accumulated. As a result, thinking has crystalized sufficiently to enable elaboration of a general theory which integrates present knowledge and serves to guide planning of future study.

The basic ideas embodied in this theory may be briefly summarized as follows: atherosclerosis is a distinct entity. It is one among the arteriosclerоses, and by far the most important. Further, it is a process separate and different from hypertension. The specific entity atherosclerosis is the chief cause of coronary disease and cerebral vascular disease. It is not simply aging or senescence. It is a disease—and therefore it is neither inevitable nor irreversible. Rather, being a disease, it is preventable and (at least up to a point) reversible, that is curable. Specifically it is a metabolic disease, in which altered cholesterol-lipid-lipoprotein metabolism plays a critical and decisive (but not exclusive) role. Without deranged lipid metabolism, clinically significant atherosclerosis would occur but rarely, particularly in middle-age, regardless of the functional status of the cardiovascular system. Finally, these atherogenic alterations in cholesterol-lipid-lipoprotein metabolism are frequently brought about by nutrition, by the habitual life-span pattern of diet. This set of concepts—the intellectual framework of reference for most current atherosclerosis research—is appropriately designated the cholesterol-lipid-lipoprotein theory of atherogenesis.

This fundamental metabolic concept of atherogenesis rests upon an extensive foundation of knowledge. It is documented by abundant data accumulated by the three major investigative approaches to the atherosclerosis problem—the clinico-pathological, the epidemiological and the animal-experimental methods of research.

Let us briefly examine the evidence from each.

The *clinico-pathological* approach is the oldest source of our knowledge concerning atherosclerosis. It is responsible for its delineation as a distinct disease entity—a delineation achieved by the pathological anatomists of the Virchow school, who recognized that the atherosclerotic plaque is unique, particularly in its massive content of cellular and extracellular cholesterol and lipids. Pathology also showed that the foam cell 'cushion', made up of one or more layers of cholesterol-lipid-laden cells (lipophages) in the intima of the

[1] Established Investigator of the American Heart Association.

artery, is the first stage histologically of the disease in man. This has been amply confirmed in animal experiments. Biochemical-pathological studies further demonstrated that the lipids of young atherosclerotic lesions exist in the same ratios to each other as in plasma, suggesting that they are derived from plasma lipids by filtration across the arterial endothelium. Such morphological-chemical observations suggested, but by themselves could not prove, that cholesterol-lipids might be key initiating factors in the pathogenesis of the lesion.

Subsequent clinical studies indicated that premature severe atherosclerosis is encountered with unusual frequency in several diseases, e.g. essential familial xanthomatosis, the nephrotic syndrome, myxedema, diabetes mellitus, which have one feature in common—the supervention of prolonged periods of hypercholesterolemia. More recently, it was definitely proved that any group of middle-aged patients with uncomplicated coronary heart disease tends to have significantly higher levels of circulating cholesterol-lipid-β-lipoproteins than a comparable clinically normal control group. While these clinical-pathological studies implicated cholesterol-lipid-lipoprotein metabolic alterations in the pathogenesis of atherosclerosis, leads on the role of diet came from other studies, animal-experimental and epidemiological.

The *epidemiological* approach, the newest source of knowledge of atherosclerosis, entails the study of disease in the population at large, rather than in individual patients and groups of sick people. Its premise is that a great deal of information on pathogenesis and etiology of disease may be obtained in the course of getting the answers to such questions as: How does coronary heart disease vary in time and in space? Is it more prevalent today than 50 years ago? Is it more frequent in some countries than others? Does ethnic origin play a role? Do males differ from females, rural from city dwellers, laborers from professional or white collar workers, etc.?

Epidemiological research does not view the collection of statistics answering such questions as an end in itself. It utilizes these data as bases of operation in the quest for solution of the decisive problem: What accounts for observed differences among population groups in morbidity and mortality due to a given disease? Epidemiology focusses on the human species in its natural environment. In the world today, and within the United States, biological and sociological environment is extremely variable as regards climate, occupation, income, diet, housing and mores. This is also true of earlier periods of man's existence. Data on these differences can be procured from archeological, anthropological and sociological sources. Field studies can supplement information already available. Thus, it is possible to accomplish extensive analyses, particularly on factors in the mode of life of peoples, and the possible relationship to patterns of morbidity and mortality. These can be particularly fruitful when skillfully cross-fertilized with clinical and laboratory research.

That area of epidemiology which studies the so-called chronic degenerative diseases, including atherosclerosis, is a young and virgin field. It has pitfalls for the unwary, but it is vigorous and has already made important contributions. As long ago as 1934, a survey of the literature showed a relationship in population groups between intake of cholesterol-fat and occurrence of atherosclerosis. In the last few years, studies on Ceylonese, Okinawans, Japanese, South Africans, Guatemalans, Costa Ricans, Dutch, English, Italians, Spaniards, Scandinavians, young American soldiers killed in Korea, etc. have demonstrated marked differences among peoples in coronary atherosclerosis, both clinical and anatomic. Further, the evidence has strongly confirmed the earlier conclusion that these differences are related to differences in life-span patterns of diet, particularly differences in the level of calorie-cholesterol-lipid intake.

Not only can a correlation be made between pattern of diet and morbidity and mortality due to this disease, but a broader generalization can now be made concerning the interrelationship among diet, plasma cholesterol-lipid-lipoprotein patterns and atherosclerosis. Population groups habitually ingesting a diet low in calories-cholesterol-lipids tend to have low plasma cholesterol-lipid-β-lipoprotein levels and little coronary atherosclerosis and ischemic heart disease. Contrariwise, population groups ingesting a diet "rich" in calories-cholesterol-lipids over the life-span tend to have high plasma cholesterol-lipid-β-lipoprotein levels and extensive coronary atherosclerosis and ischemic heart disease.

In striking confirmation of this generalization, when populations on an atherogenic diet have been compelled for a significant period of time to abandon their habitual gustatory proclivities—as was the case in Scandinavia, the Low Countries and other occupied European nations during

World War II—the tendency to high death rates from arteriosclerotic heart disease was reversed, to return again when the old diet was resumed.

Lest there be any skepticism concerning the factual bases for these theoretical generalizations, permit us to cite very briefly some United Nations data illustrating the marked differences in dietary patterns in different parts of the world. In countries like Brazil, Burma, Chile, Colombia, Egypt, Greece, Honduras, India, Indochina, Italy, Japan, Portugal, Rhodesia, Venezuela, caloric intake per person per day averages less than 2500 calories; India is the lowest—1600 calories (United States of America—3200 calories). In these countries, less than 20% of calories are of animal origin, and less than 25% are derived from fats (United States of America—41% and 40% respectively; American business and professional men are estimated to ingest fats in the amount of 58% of all calories!). In the aforementioned countries, an average of only 1–14 kg of fats and oils are consumed per person per year (Japan—1, Rhodesia—2, India—3, Brazil—6, Egypt—6, Italy—11, Greece—14, United States of America—20); milk intake is a fraction of ours (Japan—6 kg/person/year, Burma—8, Egypt—25, India—46, Brazil—80, Italy—90, United States of America—253). And these figures are an average for the entire population, rich and poor alike. The bulk of the people in these countries obviously fare even worse.

Clearly, in most of these impoverished lands, undernutrition is the cardinal overriding problem, along with its inevitable concomitant, large-scale infectious disease. Under these circumstances it is not surprising that the 'degenerative' diseases, particularly atherosclerosis, are not major public health problems. The reason is readily at hand: the socioeconomic preconditions do not exist for their emergence on a large scale (in contrast to the United States and other more prosperous countries).

Similarly, it should not be at all surprising that the commonly accepted standards for normal plasma cholesterol concentration in the United States turn out to be rather high when compared with other countries. Nor should it be unexpected that in Americans (white and Negro) plasma cholesterol levels tend to rise with each decade of life—and that this pattern is absent in the Bantu of South Africa, the Indians of Guatemala, or the poorer classes of Italy and Spain—but present in the wealthier classes of the latter two countries, who eat a diet rich in calories-cholesterol-lipids, very much as we do. (Incidentally, such epidemiological findings strongly suggest that factors such as race, nationality, climate, geography have very little to do with the case.)

At this point it might be valuable to digress from the main theme for a moment to clear up a misconception. Some workers interpret results of tracer studies on the biogenesis of cholesterol to mean that diet, particularly dietary cholesterol-lipid, is of no significance in human atherogenesis. They argue that body cholesterol originates chiefly by endogenous synthesis, and not from ingested cholesterol. Hence, they conclude, dietary cholesterol normally plays only a secondary, insignificant role in cholesterol metabolism and therefore in atherogenesis.

This line of reasoning, we submit, is inadequate factually and conceptually. It is true that the bulk of body cholesterol is endogenously synthesized. It is *not* true that cholesterol metabolism is uninfluenced by diet. All recent studies demonstrate precisely the opposite, i.e. that the pattern of diet over the life-span significantly affects cholesterol-lipid-lipoprotein metabolism, particularly patterns of cholesterolemia and lipoproteinemia. Apparently continuous high calorie-high lipid-high cholesterol intakes frequently overtax, and may alter, the organism's limited regulatory functions. Relative hypercholesterolemia and hyper-β-lipoproteinemia tend to become established, facilitating cholesterol-lipid deposition and atherogenesis in the arteries.

The contemporary American diet in particular places such a continuing strain upon the metabolic processes. This diet is pernicious first of all because it is excessive in calories-cholesterol-lipids. Moreover, recent evidence suggests that this description only partially characterizes the aberrations in American nutrition. Thus, our epidemiological studies in Chicago stimulated us to a closer examination of nutrition patterns in various strata of the United States population Our preliminary data indicate, for example, that men in the lowest income group in Chicago, the unskilled common laborers, white and Negro, have the highest middle-age specific death rates due to arteriosclerotic heart disease. This finding is currently being checked and verified in a series of further epidemiological studies. Assuming its validity *pro tem.*, it presents a significant challenge. Why—in terms of the cholesterol-lipid-lipoprotein theory of atherogenesis and its central emphasis on the role of diet—should poor laborers

have significantly higher coronary disease death rates than business and professional men? Why, moreover, should the American poor have the highest coronary disease death rates in the world, whereas the impoverished peoples of Italy, Spain, Latin America, Asia, Africa are spared?

A closer examination of the diet of the American poor may resolve these paradoxes. The fact is that the American poor, white and Negro, eat a diet unlike that of the poor elsewhere in the world. Poor people in the United States of America today differ but little from their more prosperous fellow Americans in levels of calorie-cholesterol-lipid intake. Like most Americans, they also ingest a diet high in salt and 'empty' calories, derived from highly processed, refined carbohydrate, starchy and fatty foods (e.g. sugar, candy, white bread, pastries, hydrogenated vegetable fats, lard, soft beverages, potato chips, etc.). This diet has a low ratio of essential nutrients to calories, hence—despite its obesity-producing high caloric level—it is not infrequently more or less inadequate in specific vitamins, minerals, essential fatty acids and essential amino acids.[2]

This is particularly true for the diet of the American poor, who eat significantly less fresh fruits, vegetables and lean choice meats, all expensive food items. But this pattern of dietary aberration is prevalent throughout the American population today—only more so, to an exaggerated degree and extent, among poor Americans. Thus, on closer examination, the American diet is faulty not only because it is excessive in calories-cholesterol-lipids. This concept—reinforced by recent animal experiments (in monkey, rat and chick) on the marked hypercholesterolemic effect of diets high in cholesterol-lipid and low in methionine, and by studies in man of the anti-hypercholesterolemic influence of essential unsaturated fatty acids—we designate the *dietary imbalance hypothesis* concerning the relationship of nutrition to altered cholesterol-lipid-lipoprotein metabolism and atherogenesis. According to this hypothesis, the American diet tends to be more comprehensively and profoundly characterized as an *unbalanced* diet. It is malnutrition, if malnutrition means literally bad nutrition. It is not only 'luxus' in regard to some elements, but inadequate and relatively or absolutely poor in others—hence unbalanced.

It is this acquired 'rich' and unbalanced diet which—by altering cholesterol-lipid-lipoprotein metabolism—creates the decisive prerequisites for the ready genesis of atheroscelrosis in the American population at large, and particularly perhaps in the American poor. This hypothesis, developed about a year ago on the basis of our more recent epidemiological and animal studies (see below), is being extensively explored in our Department at the present time.

In putting forward these generalizations, two additional points may be made by way of further clarification: First, it is worthy of emphasis that the contemporary American diet is a relatively recent acquisition. This is particularly true in terms of the time scale of human evolution, for many of the foods we eat today were never available to primitive man. Man has been on earth, it is estimated, some 500,000 years. Up until about 8,000 years ago he was exclusively a food gatherer. As a food gatherer, he knew nothing of such foods as cereals, fermented bread, beer, dairy foods, edible oils, spices, savouring agents. (Meats, fish, shellfish, birds' eggs, insects, grubs, wild fruits and vegetables had been available in paleolithic times, but strictly on a hand-to-mouth catch-as-catch-can basis.) The entire pattern of diet was very different from anything we are used to today. It was literally impossible (and this was true for the Eskimo as well) for a primitive food gatherer to do as we Americans do—i.e. to ingest 40–60% of his calories in the form of lipids. It was equally impossible for him to ingest a diet with a low ratio of essential nutrients to calories, since naturally occurring, unprocessed foods inevitably yield a high proportion of nutrients. Contemporary primitive peoples invariably select a highly nutritious, balanced diet, whenever it is available in nature. Self-imposed malnutrition and hidden hunger (so frequent with us) is an unknown phenomenon among food gatherers.

About 8000 years ago in the Fertile Crescent of the Middle East, man made the decisive historic leap from food-gathering to food-producing and became a farmer and a herder. He thereby created the economic preconditions both for civilization (urban life) and for the kind of diet we Americans are used to today. Although this 'rich' luxus diet became potentially available with the transition to a food-producing economy, it was in actuality not made available to the great majority of mankind who lived in poverty.

[2] In fact, as animal studies show, its high caloric level is an aggravating factor under the circumstances, since the need for several specific nutrients, particularly vitamins, tends to increase as total energy intake rises.

Only recently, in a few economically developed countries, has a 'luxus' diet become widespread among the population. The 'rich' unbalanced aberrant form that our 'modern' diet takes in the United States is a special byproduct of our national evolution, spontaneously and insidiously acquired, in the course of the mushrooming industrialization and urbanization of a vast country. In this regard it is apropos to note the extent of change in American dietary patterns just since the turn of the century. For example, Department of Agriculture data demonstrate that in 1910 fat intake averaged only about 30% of total calories in contrast to about 45% today. Further, from 1910 to 1952, intake of milk increased from 183 to 249 quarts/person/year; eggs from 293 to 395 person/year; fats and oils from 59 to 67 pounds/person/year; sugars and syrups from 87 to 107 pounds/person/year. During these four decades, intake of fruits and vegetables rose from 368 to 433 pounds/person/year; whereas intake of potatoes and cereal products declined markedly, from 213 to 103, and from 292 to 163 pounds/person/year respectively. No change occurred in meat-fish-poultry intake or in total poundage of food ingested. Thus, there was a significant alteration in composition and level of calorie intake. It is not surprising, therefore, that man as a species has not phylogenetically acquired the ability to adjust perfectly to the 'rich' unbalanced contemporary diet, since it is something 'new [that] has been added'!

In view of these historical evolutionary changes, it would obviously be intellectual myopia for scientists to assume that the 'modern' diets on which we are 'scientifically' nurtured are the best of all possible diets for overall health and longevity. And this brings us to our second point. The fact is that the science of nutrition, with all its splendid achievements, is not yet in a position fully to answer the key question, posed by our research needs in the field of chronic 'degenerative' disease: What is an optimal diet for optimal health over an optimal life-span? The fact is that the science of nutrition is a young science, even among the medical sciences. It is a science that has up to now rightly addressed itself to the prime problem of undernutrition. It has done a magnificant job defining the dietary essentials and thereby giving us the knowledge to combat acute famine, chronic semi-starvation, the avitaminoses, protein deficiencies (e.g. kwashiorkor), etc. This focus of attack has been fully sound, since the main problem for a majority of humanity was and is (right up to this moment) not malnutrition in the form of overnutrition, but malnutrition in the form of undernutrition, acute or chronic, gross or borderline.

Now, however, in view of the public health menace confronting us in the form of atherosclerosis—and the undoubted role that distorted diet plays in its genesis—nutritional science must be expected to raise its sights and assist us by devoting more of its energies to seeking out the answer to the foregoing decisive question.

In thus stressing the central relationship between nutrition and atherosclerosis, it is not our intention to assert that atherosclerosis is a dietary disease, pure and simple. To illustrate the fallaciousness of such an oversimplification of the problem, one need only consider the course of this disease in any large group of middle-aged Americans, male and female. Although ingesting similar diets, the women would have much less coronary atherosclerosis, clinically and anatomically. Among the men, a minority would exhibit demonstrable clinical signs of coronary heart disease, while a higher percentage would have gross anatomic evidence of significant coronary atherosclerosis were they autopsied. Diet alone cannot account for such sex and individual differences. It would thus appear that in population *groups* habitually 'exposed' to potentially atherogenic diets, given *individuals* may or may not be victimized by the disease, depending apparently upon an intricate interplay between diet and individual differences in genetics, heredity, sex, physical activity, fatigue, stress, other metabolic diseases, hypertension, local factors (vascular trauma, infection, allergy), etc.—factors which are accelerators or retarders.

Finally in this connection, it is important to stress that diet probably influences the development of atherosclerotic disease above and beyond its decisive effect on cholesterol-lipid-lipoprotein metabolism. Thus, nutritional state almost certainly plays a role, directly and/or indirectly, in altering the structure and function of the arterial wall and thereby establishing sites of predilection for cholesterol-lipid-lipoprotein deposition and atherogenesis. Further, diet may be a factor in the pathogenesis of hypertension, which—in the presence of alterations in cholesterol-lipid-lipoprotein metabolism—tends to accelerate and intensify atherogenesis. Even more complex interrelationships may be at work, e.g. between nutrition and function of the endocrine glands. (Discussion of hormonal influences on atherogenesis is

beyond the scope of this paper.) In addition, diet may influence several secondary processes which are very important in making atherosclerosis manifest clinically as angina pectoris or myocardial infarction. These processes include: ulceration of atherosclerotic plaques with escape of atheromatous material causing embolic arterial obstruction; increased tendency to blood clotting, facilitating thrombus formation on the surface of plaques, particularly after ulceration, with thrombotic vascular occlusion; increased capillary fragility, leading to intramural hemorrhage in plaques and arterial occlusion. As yet, little is known concerning the influence of diet and other factors on these important secondary processes. In rounding out the picture by calling attention to these complex secondary interrelationships, one must not lose sight of the basic lesson from the epidemiological data—the key role of diet in atherogenesis among peoples exerted via its effects on cholesterol-lipid-lipoprotein metabolism.

The *animal experimental* approach has contributed considerable evidence in support of the cholesterol-lipid-lipoprotein concept of atherogenesis. In order to appreciate this it is important to note that prior to 1908 many methods had been tried to reproduce the lesion of human atherosclerosis in laboratory animals. All sorts of arterial lesions resulted, but not atherosclerosis. The first success came between 1908 and 1912 at the hands of Anitschkow, Ignatowski and Chalatow who were able to induce atherosclerosis by feeding rabbits cholesterol-containing tissues or pure cholesterol in oil. This has been amply confirmed since then. In recent years atherosclerosis has been successfully induced in every one of the major laboratory animals—exclusively by procedures effecting an alteration in cholesterol-lipid-lipoprotein metabolism. In the dog, this has been accomplished by a combination of cholesterol-lipid feeding and induced hypothyroidism; in the monkey and rat by a combination of cholesterol-lipid feeding and a diet deficient in sulfhydril-containing amino acids; in the chick, hamster, guinea pig and rabbit, by cholesterol feeding alone; in geese by forced feeding. The important generalization is that these experimental procedures in all cases involved high cholesterol-high lipid dietary regimens, and induced a sustained hypercholesterolemic hyperlipemia.

Our studies, begun about 15 years ago, made use of an ominivore the chick, because results on the rabbit, a herbivore, were being severely criticized at that time as being exceptional and irrelevant to the problem in man. This criticism, it now turns out, was quite unjustified. Our concern has been with two general aspects: *1*) the role of dietary factors in atherogenesis, and *2*) the role of hormones. The general design of our experiments has been to analyze the animal's response to different atherogenic diets under conditions of controlled variations in the endogenous function of the organism. Since our interest has been in the long-term interrelation between total organism and total environment, our emphasis has been on chronic experiments utilizing intact animals.

With the demonstration that atherosclerosis could be readily induced in chicks by cholesterol feeding, experiments were quickly devised to quantitate the atherogenic process in terms of amount and duration of cholesterol feeding, degree of hypercholesterolemia, etc. Thus it was shown that within limits, there is a minimal and a maximal level of cholesterol in the diet, insofar as atherogenic influence is concerned. Levels below the minimal are ineffective, levels above the maximal have no further accelerating effect on atherogenesis. In the range between minimal and maximal levels, with all other factors held constant, the rate of atherogenesis is a function of the concentration of cholesterol in the diet.

Further, it was demonstrated in the chick, as in the rabbit, that feeding small quantities of cholesterol-lipid over long periods of time (concentrations comparable to that in the luxus American diet) produces atherosclerosis despite minimal hypercholesterolemia and no organ, skin or tendon cholesterosis—a lipid pattern similar to that seen with atherogenesis in man.

Once controlled quantitation was achieved, it became possible to analyze the long-term effects of various exogenous and endogenous factors on atherogenesis. In order to accomplish as much as possible in the shortest possible time, and thus be able to answer the many questions constantly perplexing us, cholesterol in the diet has been used at concentrations distinctly above the minimal level capable of producing atherosclerosis if fed over a longer time period.

Differences in the atherogenic activity of cholesterol were found among individual chicks in each test series. Besides, variations in atherogenic activity were revealed at different ages of the chick, between mature male and female birds, and among different breeds and strains of the species. It was therefore necessary to run matched controls in all studies.

Having established these baselines, the following more complex nutritional interrelationships were delineated:

a) Influence of Neutral Fat. With a given concentration of cholesterol in the chick diet, the level of neutral fat modified the atherogenic response. Thus, cockerels on a completely fat-free cholesterol mash diet[3] had markedly lessened cholesterolemia and atherogenesis. Hence, neutral fat in the diet appears to be necessary for development of significant cholesterol-induced atherosclerosis. The addition of 5% cottonseed oil to a cholesterol-mash diet had no demonstrable influence on atherogenesis (except in the case of pancreatectomized chicks). On the other hand, use of 20% cottonseed oil considerably aggravated cholesterolemia and atherogenesis. In addition, it resulted in hemorrhage into plaques, a phenomenon seen experimentally in only one other circumstance, viz. cholesterol feeding plus thiouracil-induced hypothyroidism. This high fat diet made the birds decidedly undernourished and puny (see below). Apparently, therefore, high neutral fat intakes, in combination with cholesterol, are particularly pernicious.

In view of recent studies on the anti-hypercholesterolemic effect of essential unsaturated fatty acids, it is noteworthy that cottonseed oil—high in linoleic acid—did not suppress hyperlipemia in cholesterol-fed cockerels. Further, no differences have been noted so far in cholesterolemia or atherogenesis in cholesterol-fed chicks given neutral fats of animal or vegetable origin (lard and cottonseed oil), or fats of different vegetable sources (linseed and cottonseed oils). It is possible that the several fatty acids exert different effects depending upon their structure, especially chain length, branching and unsaturation. In addition, the ratio (imbalance) in the diet between cholesterol and essential fatty acids may be of decisive importance. Further work is proceeding on this problem, a key one particularly in view of its possible importance clinically.

b) Influence of Undernutrition and Other Types of Dietary Imbalance. Undernutrition in the chick aggravated the cholesterolemic and atherogenic action of cholesterol. This undernutrition was induced by feeding an excessively high fat diet (see above), by 40% reduction of per diem intake of a cholesterol mash diet, or by intermittent starvation. Dietary imbalance, specifically methionine deficiency, in combination with cholesterol-oil feeding, also resulted in intensified atherogenesis. This is in accord with the results of others in rat and monkey. Choline and pyridoxine deficiency have likewise been reported to cause augmentation of cholesterol-fat atherogenicity. These findings in several species constitute experimental support for the hypothesis that cholesterol-lipid-'rich' unbalanced diets, relatively or absolutely inadequate in certain essential nutrients, may be particularly pernicious in their atherogenic potential for man. Hence, further studies are currently proceeding testing multiple deficiencies and multiple supplementations in chicks. With respect to the latter, preliminary results suggest that cholesterol-oil ingestion is minimally atherogenic when incorporated in a synthetic diet high in proteins and vitamins. This observation, if verified in subsequent experiments, poses the question whether a diet high in proteins and vitamins might be beneficial for man. If so—what vitamins, what amino acids accomplish this beneficial action? And by what mechanisms? We are currently actively engaged in following up these exciting new leads.

c) Influence of Lipotropic Factors. All careful work indicates that the so-called lipotropic factors are without effect on cholesterolemia and atherogenesis when added to nutritionally adequate rations supplemented with cholesterol.[4] This is true for intact and for depancreatized chicks. The substances tested by us included choline, inositol, pancreatin, activated whole pancreas, anti-fatty liver factor, tocopherols, vitamin B_{12}, lipocaic, lecithins, etc. These negative results are completely in agreement with those of most other workers studying other laboratory animals and man. Thus, there is no research basis to support the widely advertised claims of some pharmaceutical houses that proprietary lipotropic preparations are of value in the prevention and treatment of human atherosclerosis.

d) Influence of Sterols and Brain Extracts. More encouraging results have recently been obtained with defatted brain extract preparations, as well as with plant sterols and other sterols closely resembling cholesterol. These materials, when fed to laboratory animals together with

[3] Commercial chick mash used in our laboratory contains about 4% total fats. Fat-free mash was especially prepared utilizing extraction procedures.

[4] The fact that lipotropic factor supplementation of a generally *adequate* diet failed to influence atherogenesis, in no way contradicts other observations that *deficiencies* in some of these factors augmented atherogenesis (see above).

cholesterol, apparently prevented the gastrointestinal absorption of cholesterol, thereby inhibiting hypercholesterolemia and atherosclerosis. Unfortunately, recent results indicate that some of these sterols may themselves be absorbed, with resultant atherogenesis. Hence, these materials must still be considered experimental. Final judgment of their clinical efficacy must be withheld pending further work.

e) Diet-Induced Reversal of Atherosclerotic Lesions. At the onset of this paper it was stated that atherosclerosis is a reversible disease. This conclusion is based on extensive data collected in rabbit, chick, dog and man. Several years ago in our laboratory, an experiment was carried out in which chicks were fed a cholesterol-oil mash for several weeks, with development of extensive aorta lesions. Cholesterol-oil feeding was then stopped and the birds restored to ordinary chick mash. During subsequent weeks, definitive extensive regression of lesions was readily demonstrable. In later studies it was shown that such diet-induced reversal of lesions also occurred in the coronary vessels.[5]

More recently, several studies have been carried out on various factors influencing the regression of aorta and coronary lesions which supervenes following upon cessation of cholesterol-oil feeding. To mention two of these which appear to be most significant, cockerels with cholesterol-induced lesions exhibited minimal regression when fed ordinary mash in an inadequate amount (60% of normal). This observation on the deleterious effect of undernutrition is in accord with other findings (see above).

Further, when insulin was given in the period following cessation of cholesterol-oil feeding, the usual regression of lesions in the coronary vessels failed to occur. This observation on the atherogenesis-potentiating effect of insulin is in accord with other data from our laboratory. It poses fundamental problems concerning the possible role of exogenous insulin, given over long periods of time for the treatment of human diabetes mellitus, in the premature severe atherogenesis characteristically developing in such patients. This whole problem, which is beyond the scope of this discussion, is currently under active exploration in our laboratory.

Finally, it should be noted in passing that restoration of a normal diet is not the sole means of reversing cholesterol-induced atherosclerosis in chicks. For example, estrogenic hormone exhibition induces complete reversal of coronary lesions in cockerels, despite continued feeding of an atherogenic diet during the period of hormone administration.

From this survey, it is apparent that animal experiments have contributed a great deal to our understanding of the relationship between nutrition and atherogenesis. While many difficult specific questions remain to be answered and the overall edifice of our knowledge is still imperfect, it would seem valid to conclude that evidence from animal studies—along with that from clinical and epidemiological research—supports the concept that atherosclerosis is essentially a problem of cholesterol-lipid-lipoprotein metabolism in which nutrition plays a key role.

Based on this overall conclusion, the question arises: Is medical science in a position to advise concerning dietary prophylaxis and therapy against atherosclerosis in man? Obviously, the fact that species differences do exist demands that all animal observations of significance be reexamined in man. Obviously, too, data available to date from clinical and epidemiological studies do not permit an absolute answer to the foregoing question. Nonetheless, it would seem justified—particularly in view of the tremendous toll constantly being taken by this disease—to advance certain tentative ideas, based on generally sound nutritional and hygienic principles which can do no harm. For, under the circumstances, does not the burden of proof rest with those who say 'do nothing'?

Certainly 'doing something' about dietary prophylaxis against atherosclerosis does not mean proposing that the American people adopt the totally inadequate chronic starvation diets of the Bantu in South Africa or the masses of India. In terms of its consequences in malnutritional and infectious disease, this would be literally 'throwing out the baby with the bath.' Similarly, it is equally unnecessary and unwise to recommend adherence to the 'rice diet' by the American population, who would not tolerate it anyway, and with good sense.

If our overall theoretical conclusions concerning the relationship of nutrition to atherogenesis are sound, proposals for prophylaxis should in-

[5] Based on other work in this laboratory, demonstrating that different laws govern atherogenesis in different vascular beds, it is now recognized that the experimentalist cannot confine his studies to the aorta, but must examine other vascular beds as well, particularly the coronary vessels.

volve simple rational adjustments of contemporary patterns of diet. The objective should be to correct the tendencies to excessive intake of calories-cholesterol-lipids and to inadequate intake of essential vitamins-minerals-amino acids–unsaturated fatty acids.

The accomplishment of this goal is really quite feasible from every point of view, including palatability and enjoyment of eating. It simply involves the substituting of broiling of meat for deep fat frying and roasting; abandonment of such habits as pouring gravies on meats, and sauces and dressings on vegetables and salads; and taking a leaf from the book of France by substituting fresh or cooked fruit for such customary American desserts as pie a la mode, ice cream, pastries, etc. These measures, together with a general increase in consumption of fruits, vegetables and whole grain products (instead of bleached white flour) are probably about all that is needed. They would automatically cut down calories and therewith obesity, significantly raise the ratio of essential nutrients to calories, reduce the percent of total calories in the form of fat from the present 40–60% to a reasonable 25–30%, and thus solve most of the problems of the present day 'rich' and unbalanced American diet. Obviously, this is neither a final nor a complete answer to the problem of the prophylaxis of atherosclerosis. Knowledge does not permit us to be so definitive. But the above set of suggestions almost certainly constitutes a healthy step in the right direction.

As for dietary therapy, no definitive study is as yet in the literature on this subject. One report is unfortunately inadequately controlled. Nevertheless, here again it would seem to be that the foregoing regimen is in order and that the burden of proof rests with those who say 'do nothing', particularly in view of known facts concerning prognosis in patients with clinically significant atherosclerosis.

Whatever the final judgment concerning the foregoing tentative suggestions on prophylaxis and therapy it would seem valid to conclude that significant progress has been made toward a scientific understanding of the role of nutrition in this disease. Almost certainly, our general conceptual framework of reference, the cholesterol-lipid-liproprotein theory, is sound. The goal is perhaps in sight, but it has not yet been reached. Much remains to be done on the part of many, from multiple cooperating disciplines, working together in the best traditions of scientific objectivity, perseverance and enthusiasm, based on the road already traversed.

It is a pleasure to acknowledge the contribution to this research program over the years of our former associates Dr. D. V. Dauber (deceased), L. Horlick and S. Rodbard. These investigations were successfully accomplished only by virtue of the excellent cooperation and assistance of the technicians of the department's atherosclerosis research team.

This research program was made possible principally by grants-in-aid awarded by the Life Insurance Medical Research Fund, the National Heart Institute of the Public Health Service, the American Heart Association, the Chicago Heart Association and the Michael Reese Research Foundation. It was supported by many local benefactors and organizations and by several pharmaceutical houses.

REFERENCES[6]

1. Katz, L. N. and J. Stamler. *Experimental Atherosclerosis*. Springfield, Ill.: Thomas, 1953.
2. Stamler, J., L. N. Katz and R. Pick. *Nutritional-Endocrinological Interrelationships in the Pathogenesis of Hypertension and Atherosclerosis Over the Life Span*. Fourth Annual Report on Stress. Selye and Heuser (editors). Montreal: Acta, 1954.
3. Stamler, J., L. N. Katz, R. Pick and S. Rodbard. *Recent Progr. Hormone Research* 10: 401, 1955.
4. Katz, L. N., J. Stamler and R. Pick. Symposium on Atherosclerosis. Washington: National Academy of Sciences–National Research Council, Publication, No. 338, p. 236, 1954.
5. Katz, L. N. *Ann. Int. Med.* 43: 930, 1955.
6. Katz, L. N., R. Pick and J. Stamler. *Mod. Concepts Cardiovas. Dis.* 23: 239, 1954.
7. Katz, L. N. *Circulation Res.* 4: 123, 1956.
8. Katz, L. N., J. Stamler and R. Pick. *Lancet* (in press).
9. Stamler, J. *Nebraska Med. J.*, 41: 75, 1956.
10. Katz, L. N., J. Stamler and R. Pick. *J. A. M. A.* 161: 536, 1956.
11. Katz, L. N., J. Stamler and R. Pick. *New England J. of Med.* (in press).

[6] References to work by others will be found in the first and last references.

BIOCHEMICAL ASPECTS OF ATHEROSCLEROSIS

CHRISTIAN B. ANFINSEN, JR.

From the National Heart Institute, National Institutes of Health, Bethesda, Maryland

AS DR. KATZ EMPHASIZED during his discussion, atherosclerosis constitutes one of the most serious medical problems facing mankind today. Recently the study of the etiology of this disease has been intensified by the development of a considerable amount of interest among biochemists and physiologists. During this short discussion, I would like to review for you some of the approaches which are now being made in various laboratories and to summarize briefly a few of the specific advances in knowledge that have been made. The material I shall cover has been belabored considerably recently among specialists in this field of investigation and to those in the audience who have heard similar summaries, I must apologize in advance for what will necessarily be a superficial and somewhat speculative presentation. I must further ask you to excuse the rather strong emphasis on fat metabolism which will color my remarks. Properly, we should also consider the many important studies now in progress on cholesterol metabolism, on the collagenase and elastase systems, and on other equally active fields of research. However, you will appreciate that time is limited.

It has been clear for many years that there exists some relationship between the occurrence of atherosclerosis and the processes involved in fat transport and metabolism. Such a relationship was first suspected on the basis of pathological studies on tissues which demonstrated the accumulation of lipoidal materials, particularly cholesterol, in atheromata. This relationship was further strengthened by the increased awareness of the coincidental occurrence of atherosclerosis and of metabolic diseases involving aberrations in fat metabolism, such as diabetes and nephrosis.

A large segment of the modern approach to the study of the atherogenic process stems from the fact that lipids exist in animal tissue and fluids in the form of lipoprotein complexes and that the metabolism of lipids in an aqueous environment is made possible by such a combination. Cellular lipoproteins have not yet been properly characterized. However, we now recognize three major forms of circulating lipid. The first of these is the chylomicron, which consists almost entirely of neutral fat but which contains small and probably very significant amounts of cholesterol, phospholipid and protein. These chylomicra appear in the lymphatic drainage of the intestine following a fatty meal. The second and third classes are the alpha- and beta-lipoproteins, whose chemical architecture is somewhat better defined. Our present understanding of these latter molecules has been greatly advanced by the introduction of elegant physical techniques by Pedersen (1) and by Gofman and his collaborators (2), as well as by chemical fractionation methods developed by Cohn, Edsall and Oncley (5) and their collaborators and applied by Russ, Eder and Barr (4) and others. Both statistical (5) and biochemical (6, 7) studies now indicate strongly that one profitable approach to the understanding of atherosclerosis lies in the further understanding of the physiological control underlying the production, transport and destruction of these molecules in body fluids.

Figure 1 shows an extremely schematic representation of some of the unusual characteristics of the beta- and alpha-lipoprotein molecules (8). The beta-lipoprotein is an extremely large molecule containing approximately 75% fat, which is partitioned among the various forms of lipids as shown. Recent studies by Avigan, Steinberg and Redfield (9) suggest the presence of only a single large peptide chain containing an N-terminal glutamic acid. The amount of protein in the molecule is sufficient to cover only one-third to one-half of the surface area. The alpha-lipoprotein is considerably smaller, contains about 35% lipid, and also appears to be a single chain, terminating in aspartic acid (9). Although the amino acid compositions of the alpha- and beta-lipoproteins are very similar, there seems little doubt that the protein moieties of these large complexes represent two different chemical components, chemically, physically and im-

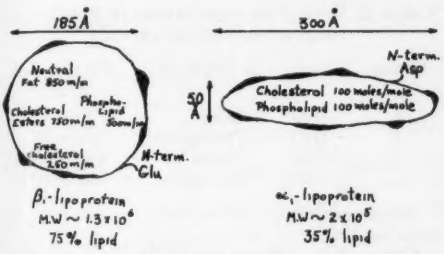


FIG. 1. A schematic summary of the chemical compositions and approximate sizes and shapes of the α and β-lipoprotein molecules.

munochemically (personal communication from R. K. Brown).

Figure 2 gives what we might call a high magnification of the surface cross-section of a beta-lipoprotein molecule. This figure is included simply to help to summarize some of the further details of its chemical peculiarities. The molecule is highly permeable to water and, as has been shown by the use of isotopic tracer techniques, is free to exchange its phospholipid and free cholesterol constituents at a considerable speed. Neutral fat and esterified cholesterol molecules, on the other hand, are much more slowly exchangeable. The significance of these qualitative observations is still quite obscure but they suggest a protein-stabilized neutral fat-esterified cholesterol matrix with ideal fat transporting properties.

A major line of investigation now being carried out in several laboratories is concerned with the role of lipoproteins in fat transport and mobilization. Recent studies (unpublished) by Fredrickson and Havel, for example, strongly suggest that the alpha- and beta-lipoprotein moieties constitute two separate and distinct metabolic pools of circulating lipid that may mediate quite different physiological processes.

A major interest in several laboratories including our own has been in connection with the mechanisms involved in the homeostatic regulation of fat transport. These studies stem from the earlier observations by Hahn (10), Anderson and Fawcett (11) and others (12, 13) that alimentary lipemia was rapidly 'cleared' by the administration of heparin to experimental animals. These early observations have now evolved into the clearcut delineation of an enzyme system which has the specific properties of digesting the triglycerides of lipoproteins. In contradistinction to ordinary pancreatic lipase this enzyme system is active only on lipoprotein-bound lipids and has, therefore, been given the name 'lipoprotein lipase' by Korn (14–16), who has recently greatly extended our understanding of its nature.

Table 1 summarizes some of the properties of this enzyme. The enzyme attacks the triglycerides of chyle and low-density beta-lipoproteins at a rapid rate. Further, it can attack ordinary triglyceride emulsions when these have been 'activated' by combination with the alpha-lipoprotein fraction of fat. Table 1, part B, shows that the reaction proceeds with the production of fatty acids and glycerol and that the

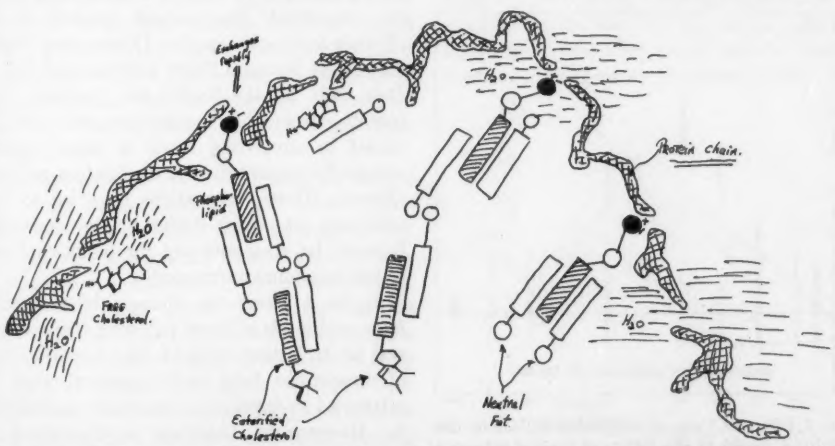


FIG. 2. A 'cross-section' of the β-lipoprotein molecule, suggesting the relatively greater exchangeability with serum and tissues of free cholesterol and phospholipid in relation to neutral fat and esterified cholesterol.

TABLE 1

A. Substrate

1) Chylomicrons, low-density β-lipoproteins
2) Synthetic 'chylomicrons'
   Triglyceride emulsion + α-lipoprotein → 'activated' triglyceride

B. Hydrolysis

'Activated' triglyceride

$$\xrightarrow[\text{albumin or } Ca^{++}]{\text{Lipoprotein lipase}}$$

Complexed fatty acids + glycerol (+ mono & di-glycerides during early stages)

over-all reaction requires the presence of a fatty acid binder which, under physiological conditions, has been demonstrated by Gordon and his colleagues (17, 18) to consist of serum albumin. On the basis of the most recent evidence, lipoprotein lipase appears to contain heparin or a heparin-like substance as part of its structure since it is inactivated both by protamine (13) and by treatment with a rather specific heparinase isolated from heparin-degrading bacteria (19).

The relation of the lipoprotein lipase system to normal fat metabolism and to atherosclerosis is at present mainly based on circumstantial evidence. Havel, Gordon, Fredrickson and Bragdon (unpublished observations) have, for example, recently studied two siblings with idiopathic hyperlipemia, whose plasmas contained no lipoprotein lipase following heparin administration. The chylomicron-like lipid in their plasma was, however, quite susceptible to lipoprotein lipase attack *in vitro*. Thus, these patients might conceivably represent instances in which the production of the protein moiety of this enzyme is extremely deficient or absent.

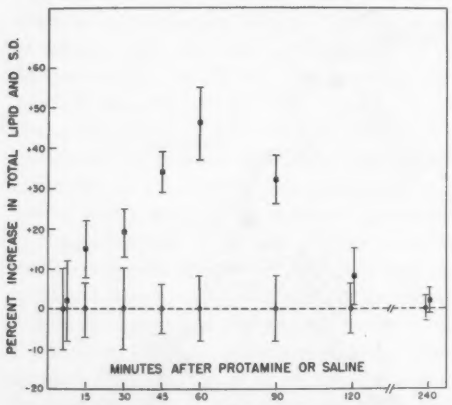


FIG. 3. Effect of 1 mg. of protamine sulfate on the 'total lipid' levels of the serum of fasting rats as a function of time. ● represents mean levels in controls. ■ represents mean levels after protamine. Standard deviations are also shown.

TABLE 2. EFFECT OF CARBOHYDRATE FEEDING IN PROTAMINE-INDUCED LIPEMIA

Group	Fed*	Injected	Total Lipid (Units)
1	H_2O	Saline	4780±410
2	H_2O	Protamine†	7450±700
3	CHO	Saline	4030±557
4	CHO	Protamine	5000±1100

Blood samples taken for Nicloux analysis 1 hr. after injection.

* Given 1 hr. before injection by stomach tube and again immediately after injection.

† 1 mg in saline (1 ml).

Further indirect evidence can be obtained from studies such as those shown in figure 3. These studies derive from the observation that lipoprotein lipase is extremely sensitive to low levels of protamine. Upon the injection of protamine into normal fasting rats, Bragdon and Havel (20) have demonstrated a rapid, transient increase in the total plasma lipids as compared to rats given saline alone. The lipemia-producing effects of protamine are, however, prevented by previous carbohydrate feeding of the rats as shown in table 2 (unpublished observations). I will defer mentioning the conclusions that can be drawn from these experiments until we have considered another aspect of the problem, which bears a close relation to them.

As was indicated in the table above, showing the mechanism of action of lipoprotein lipase, the important physiological product is the albumin-fatty acid complex. Gordon and Cherkes (21) at the National Heart Institute and Vincent Dole (22) at the Rockefeller Institute have recently shown that unesterified fatty acid contained in circulating blood is taken care of, essentially quantitatively, by binding to serum albumin. These observations have led to some extremely interesting studies on the relationship between fat transport and the nutritional state of the individual under study.

Figure 4 shows the changes in unesterified fatty acid levels of blood following the feeding of oral fat to fasting subjects. The increased levels of unesterified fatty acids observed were first attributed to digestion of the orally administered fat. However, in subsequent experiments it was found that a similar increase took place during fasting alone, without fat administration. This observation suggested that the increase in un-

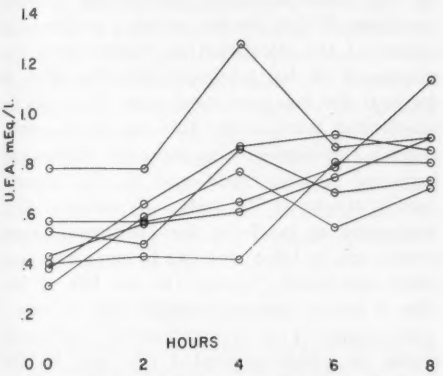


FIG. 4. Response of unesterified fatty acids to oral fat in 7 normal young men.

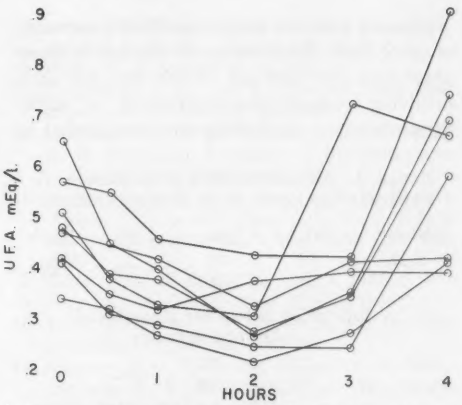


FIG. 5. Response of unesterified fatty acids to oral glucose in 8 normal young men.

esterified fatty acids might rather be due to a deficiency in circulating calories, specifically carbohydrate. That this conclusion was correct was shown by experiments summarized in figure 5, in which the unesterified fatty acid levels were found to drop following glucose feeding to fasting subjects. It was further observed that the initial decrease due to glucose was followed by the large rise in fatty acid levels. Since epinephrine is known to be released in response to the transient hypoglycemia which follows several hours after the feeding of glucose, similar experiments were carried out on fasting subjects to whom epinephrine was administered. These experiments showed, as indicated in figure 6, that this hormonal agent could, indeed, increase unesterified fatty acid levels. Since glucose tends to depress fatty acid levels, and since epinephrine mediates an increase in blood glucose, one must conclude that epinephrine has an effect on unesterified fatty acid that is distinct from its effect upon glucose levels.

Further investigations based on these studies have been carried out on the arteriovenous differences across various localized areas in the body during caloric modification. Table 3 shows, for example, the results of direct measurements of the AV differences, across the myocardium, of fatty acids and oxygen. In the fasting subject the myocardium appears to derive a major share of its energy from unesterified fatty acids, often over 70% in well-fasted subjects. Of particular interest is the finding that the fatty acid level entering the myocardium is markedly decreased following glucose administration, and that simultaneously the extraction of UFA by myocardium ceases. It is known from the work of Bing (23), Goodale (24) and their colleagues that under these circumstances the myocardium extracts large amounts of glucose. The counteracting effect of glucose on FA utilization, in short, appears not to take place in the metabolizing tissue itself, but directly or indirectly at the site of liberation of UFA into the blood.

Figure 7 shows a schematic summary of a number of such experiments in which AV differences were measured across different metabolizing regions of tissue. As is shown in this figure, some areas show positive, and others negative, AV differences, the latter being the case for areas which might be considered more highly implicated as fat depots. Thus the saphenous vein, draining the adipose-rich leg area is higher, the myocardium and liver lower, and the brain unchanged in comparison to arterial blood.

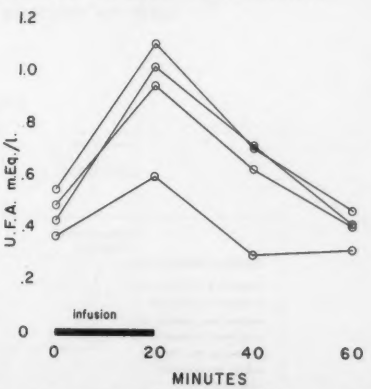


FIG. 6. Response of unesterified fatty acids to epinephrine in 4 normal young men.

Figure 8 presents a very speculative summary of all of these observations. At the top is shown absorption from the gut of fats into the chyle involving perhaps the interaction of alpha-lipoprotein as a combining site as suggested by the triglyceride activating studies with lipoprotein lipase. Within the box is drawn a schematic picture of the circulation as viewed from the standpoint of fat transport. On the right is depicted the transport mechanism for lipid as unesterified fatty acids. This transport mechanism is visualized as being under the influence of hormonal control, conditioned by the glucose level of the blood. We tentatively visualize this mechanism as involving the lipoprotein lipase system and as being sensitive to protamine and other anti-heparin agents. On the left of the slide is drawn a second possible type of mechanism suggested by the experiments I mentioned earlier on protamine-treated rats and on the hyperlipemic patients unable to produce lipoprotein lipase. In this mechanism the transport of fat is brought about by transport as lipoproteins. Such a mechanism might only be seen under circumstances where neither glucose nor fatty acid is adequate to maintain the calorie requirements of the metabolizing tissues. It is tempting to speculate further whether starvation lipemia might not be included in this second type of transport mechanism.

TABLE 3. ARTERIOVENOUS DIFFERENCES IN UNESTERIFIED FATTY ACIDS (CORONARY SINUS)

Patient	Art.	Ven.	Diff.	% O_2
S.M.	.94	.76	.20	42
	.98	.75		
Repeat 30 min. after glucose and insulin intravenously				
	.32	.32	none	
	.30	.29		

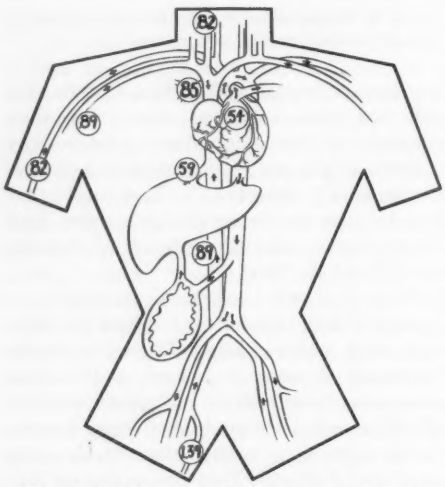


FIG. 7. Arteriovenous differences in the level of unesterified fatty acids in the fasting normal subject.

Before leaving the subject of fatty acid transport, we should mention the great interest being shown at present in the relative effects of saturated and unsaturated fatty acids on lipid transport in general. The striking effects of the more highly unsaturated fats in lowering the blood cholesterol and low-density lipoprotein levels, as shown by Kinsell (25), Ahrens (26) and others, suggest that unsaturation may have an extremely important role in lipid metabolism.

In closing, I should like to emphasize that the

SCHEME FOR TRIGLYCERIDE TRANSPORT AND METABOLIC HOMEOSTASIS

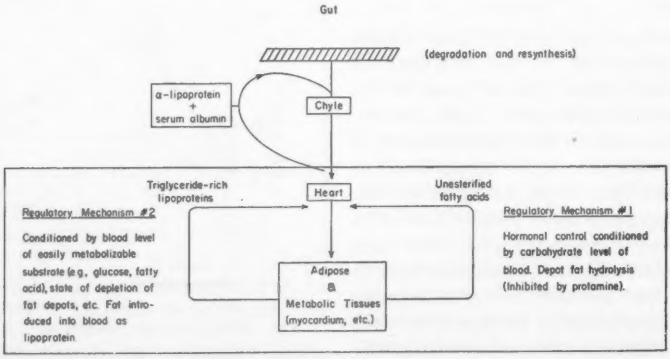

Gut
(degradation and resynthesis)
α-lipoprotein + serum albumin
Chyle
Triglyceride-rich lipoproteins
Heart
Unesterified fatty acids
Regulatory Mechanism #2
Conditioned by blood level of easily metabolizable substrate (e.g., glucose, fatty acid), state of depletion of fat depots, etc. Fat introduced into blood as lipoprotein
Regulatory Mechanism #1
Hormonal control conditioned by carbohydrate level of blood. Depot fat hydrolysis (inhibited by protamine).
Adipose & Metabolic Tissues (myocardium, etc.)

FIG. 8.

study of atherosclerosis must go a great deal further than the cholesterol molecule alone. Those of us who are involved in this complex and fascinating problem must also aim toward a greater integrated understanding of the general phenomena underlying triglyceride transport and metabolism. The aberrant pattern of hormonal interactions so evident in diabetes is almost certain to find its parallel in the case of atherosclerosis, a disease that is so obviously linked with the metabolism of fat.

REFERENCES

1. PEDERSEN, K. O. *Ultracentrifugal Studies on Serum and Serum Fractions.* Uppsala, Almqvist and Wiksell, 1945.
2. GOFMAN, J. W., F. LINDGREN, H. ELLIOTT, W. MANTZ, J. HEWITT, B. STRISOWER AND V. HERRING. *Science* 111: 166, 1950.
3. EDSALL, J. T. In: *Advances in Protein Chemistry*, New York: Acad. Press, 1947, volume III.
4. RUSS, E. M., H. A. EDER AND D. P. BARR. *Am. J. Med.* 11: 468, 1951.
5. KEYS, A. *Minnesota Med.* 38: 28, 1955.
6. ANFINSEN, C. B. *Minnesota Med.* 38: 767, 1955.
7. *Symposium on Atherosclerosis.* Washington, D. C.: Nat. Acad. of Sciences-National Research Council, 1955.
8. ONCLEY, J. L., F. R. N. GURD AND M. MELIN. *J. Am. Chem. Soc.* 72: 458, 1950.
9. AVIGAN, J., D. STEINBERG AND R. R. REDFIELD. *Biochim. et biophys. acta* 20: 557, 1956.
10. HAHN, P. F. *Science* 98: 19, 1943.
11. ANDERSON, H. G. AND B. FAWCETT. *Proc. Soc. Exper. Biol. & Med.* 74: 768, 1950.
12. GRAHAM, D. M., T. P. LYON, J. W. GOFMAN, H. B. JONES, A. YANKLEY, J. SIMONTON AND J. WHITE *Circulation* 4: 666, 1951.
13. ANFINSEN, C. B., E. BOYLE AND R. K. BROWN. *Science* 115: 583, 1952; BROWN, R. K., E. BOYLE AND C. B. ANFINSEN. *J. Biol Chem.* 204: 423, 1953.
14. KORN, E. D. *J. Biol. Chem.* 215: 1, 1955.
15. KORN, E. D. *J. Biol. Chem.* 215: 15, 1955.
16. KORN, E. D. AND T. W. QUIGLEY, JR. *Biochim. et biophys. acta* 18: 143, 1955.
17. GORDON, R. S., E. BOYLE, R. BROWN, A. CHERKES AND C. B. ANFINSEN. *Proc. Soc. Exper. Biol. & Med.* 84: 168, 1953.
18. GORDON, R. S. *J. Clin. Invest.* 34: 477, 1955.
19. KORN, E. D. AND A. N. PAYZA. *Biochim. et biophys. acta* 20: 596, 1956; KORN, E. D. *Science* (in press) 1956.
20. BRAGDON, J. AND R. HAVEL. *Am. J. Physiol.* 177: 128, 1954.
21. GORDON, R. S., JR. AND A. CHERKES *J. Clin. Invest.* 35: 206, 1956.
22. DOLE, V. *J. Clin. Invest.* 35: 150, 1956.
23 BING, R. J. *Circulation* 12: 635, 1955.
24. GOODALE, W. T. AND D. D. HACKEL. *Circulation Res.* 1: 511, 1953.
25. FRISKEY, R. W., G. D. MICHAELS AND L. W. KINSELL. *Circulation Res.* 12: 492, 1955.
26. AHRENS, A. H. *J. Clin. Invest.* 34: 918, 1955.

DIETARY ASPECTS

FREDRICK J. STARE

From the Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts

IN TERMS OF FREQUENCY and seriousness, atherosclerosis, notably of the coronary and cerebral arteries, is the most important of the cardiovascular diseases. It is also the cardiovascular disease in which nutrition consideration appears to be of greatest significance.

There is evidence suggesting that many dietary factors may be involved in the enigma of atherosclerosis. Fat, protein, carbohydrate, cholesterol, choline, pyridoxine, organic sulfur—all of these factors have been implicated, and perhaps through deranged metabolism, other vitamins and minerals may be involved. Caloric balance and exercise may also be factors of importance.

The field is a large one, and in a paper of reasonable brevity only certain areas of research can be selected for consideration. Much of what will be reported in this discussion is work from our own laboratories performed by a number of associates and students, particularly Drs. G. V. Mann, O. W. Portman, L. C. Fillios, S. B. Andrus, E. Y. Lawry, W. R. Waddell, R. P. Geyer and D. M. Hegsted.

Perhaps the major interest in nutrition today is concerned with the possible relationship between dietary fat and the development of atherosclerosis. This interest has been stimulated by evidence accumulated over the past 25 years by a number of investigators from many parts of the world (1–4). The evidence consists of clinical observations in times of famine, epidemiologic studies and, more recently, some degree of planned investigative work in both animals and in man. Since most of you are probably familiar with much of this work, it is only necessary to state that there appears to be a positive correlation between total fat intake and incidence of coronary heart disease, level of serum cholesterol, and prevalence and extent of atherosclerosis at autopsy. Although the evidence for this correlation is less than might be desired, an elevation of serum cholesterol seems to be a causative factor in the development of atherosclerosis in man. In the experimental production of this condition in a variety of species, such relationship between hypercholesterolemia and atherosclerosis seems quite certain.

Most of you are also familiar with the work of Gofman and his associates (5) which purports to show that the serum levels of lipoprotein are more closely correlated with the development of atherosclerosis than are the serum levels of cholesterol. The evidence in this regard is certainly not conclusive and there are findings to the contrary but, since cholesterol is one of the constituents of the lipoproteins in blood, both determinations should be relevant. Regardless of the ultimate value of lipoprotein measurements in relation to atherosclerosis, the work of Gofman and his colleagues has been highly stimulating.

Concerning epidemiological data, it should be emphasized that, while this information has accumulated over a period of years, there is still not enough known about the interrelation of dietaries and the actual prevalence of atherosclerosis among different human societies. Much more evidence of this type needs to be gathered. At least three types of observations should be made in such studies to make them reasonably complete:

1. Dietary Information—the nature of the habitual diet with particular attention to the daily caloric, protein and fat intake (including the type of fat).

2. Blood Chemistries—to date these have been confined to measurement of levels of serum lipids, with attention to the lipoproteins, total cholesterol and phospholipids, but other chemical measurements may be added.

3. Autopsy Data—the extent of atherosclerosis determined by direct observation of tissues from subjects representing a span of ages.

The practical obstacles to *planned* human experiments intended to relate these factors are considerable; thus, more thorough studies of these matters utilizing natural environments should be done. In the meantime, it is necessary to be skeptical of conclusions drawn from impressions and inadequate observations. Those of you who have had experience in taking diet his-

tories know the difficulties and time involved in getting reliable information, and when there are superimposed language and cultural barriers the difficulties are multiplied.

Our attempts at epidemiologic studies of these problems have been carried out in collaboration with Dr. Nevin Scrimshaw and his colleagues of the Institute of Nutrition of Central America and Panama, and with Dr. Bruce Nicol of the Federal Medical Department of Nigeria. We also have one study in Boston underway involving second-generation Italians employed at the Hood Rubber Company.

The Guatemalan (6) and Nigerian (7) studies gave essentially the same findings; namely, rural subjects living on diets much lower in fat than typical American diets had much lower levels of serum cholesterol than Americans of the same sex and age. Strangely, there was little difference in the levels of the beta lipoproteins, particularly those of the S_f 12–20 class. Urban Guatemalans consumed more fat and their level of serum cholesterol was closer to that of the Americans. The proportion of the daily caloric intake provided by fat among the rural subjects studied varied from 8 to 15%; among the urban Guatemalans it averaged 36%, and among the urban Americans, 40%.

Although many investigators have emphasized the importance of differences in fat intake, it should be kept in mind that there are many *other* differences in these diets—among them, the type of fat and carbohydrate, the quantity and quality of protein, and the vitamin and mineral content. In addition, there are differences in the relative body weights, and the amount and type of activity of the subjects. Many of these items have been at times suspected of influencing cholesterol metabolism.

Is there less atherosclerosis in ethnic groups with lower levels of serum cholesterol? We have no first-hand data on the Nigerians, but reports in the literature from the Gold Coast where the cultures are supposedly similar to Nigeria state that the incidence of atherosclerosis is extremely low—less than 1% in 3600 autopsies (8). Other studies with African Bantus have shown that severe atheromata and coronary artery disease are very infrequent (9). Dr. Tejada of INCAP and Dr. Gore of our Department have specimens from over 100 autopsies performed on Guatemalans and have found very little atherosclerosis in rural subjects.

Keys has emphasized the relationship between the total fat intake and atherosclerosis. This consideration undoubtedly deserves emphasis, since very low fat diets will lower the serum cholesterol in test subjects; however, such subjects usually also lose weight. It seems, however, that too much emphasis upon the fat level may cause us to lose sight of other possibly important variables. Although Keys believes that caloric intake per se is not a variable of importance, the evidence supporting that view is incomplete, and there is some evidence to the contrary. Possibly rates of energy expenditure relative to the caloric intake are important.

Another dimension to the problem of dietary fat and its role in atherogenesis has been added as a result of the observations of Kinsell (10), Ahrens (11) and Beveridge (12), that high intakes of certain vegetable fats may also be effective in lowering the blood cholesterol. The evidence would indicate that the relatively unsaturated vegetable fats are the effective ones. In this connection, two studies from our laboratory are of interest. Some years ago, Dr. Hardinge made some comparative studies of 'pure' vegetarians, lacto-ovo-vegetarians, and nonvegetarians (13). Serum levels of cholesterol of the adult vegetarian groups were lower than adult nonvegetarian groups, the pure vegetarians having the lowest values, despite the fact that 35% of their total calorie intake came from fat, of course all vegetable fat.

Recently, we studied a group of men with relatively high levels of serum cholesterol and lipoprotein while they received approximately 1000 calories per day of a peanut oil emulsion. Their diets were not formula-type diets, as other workers have used, but consisted of common foods, though varied, so as to eliminate most of the visible fat and reduced in quantity, so that the subjects did not gain weight during the four-week period that the 1000 calories of fat emulsion were consumed. Serum cholesterols were markedly lowered in the first week but later returned to original levels. There were much larger changes in the serum lipoprotein levels and in the distribution of the serum lipoproteins. By the end of the four weeks, 18 of 24 subjects had reduced their levels of the S_f 12–20 beta lipoproteins an average of 50%. We do not know what the data mean and, as yet, the evaluation has not been completed. It is unlikely that any such dietary regimen would be an effective one or an acceptable one for patients with high cholesterol levels.

Some months ago, my colleague, Dr. William

Waddell, had occasion to cooperate with Dr. Walter Lever in a study of the disappearance of infused fat from patients ill with diseases characterized by a persistent elevation of certain of the blood lipids, namely, idiopathic hyperlipemia and primary hypercholesteremic xanthomatosis. Generally, such patients are on a low fat diet, but, even so, some of the blood lipids remain markedly elevated and atherosclerosis may be a common and early complication.

As was to be expected, infused fat in patients with such diseases was slow in leaving the blood stream. In patients without this disorder, the blood lipids returned to a pre-infusion level in three to six hours following the infusion, but here they took 12 to 18 hours to get back to pre-infusion levels. These patients generally have a high serum cholesterol, and much to our surprise, this was rather markedly decreased following infusion of a fat emulsion. The decrease was evident at the termination of the fat infusion and became more striking as the neutral fat was cleared.

Perhaps the magnitude of this decrease following a single infusion of fat emulsion may best be indicated by a few specific examples (14). Four patients with primary hypercholesteremic xanthomatosis had an average serum cholesterol of 556 mg % prior to infusion of fat. Twenty hours after one fat infusion, the average was 436, a decrease of 20%. Nine patients with idiopathic hyperlipemia had serum cholesterols averaging 579 mg %, and 20 hours after a single infusion of fat emulsion to each, this average decreased to 482 mg %.

Naturally, we were interested in seeing what would happen following several daily infusions of fat emulsion. Here, a specific example will best tell the story. A patient with idiopathic hyperlipemia and a serum cholesterol that had consistently run above 600 mg % for a long time was infused with fat for 7 consecutive days, and the serum cholesterol values in milligrams per cent 20 hours after each of these 7 infusions are as follows: from over 600 down to 560, then to 475, 410, 370, 320, 300, 260—a decrease of 60% in 7 days.

For fat emulsions given by vein to result in a reduction of the serum cholesterol seems somewhat of a paradox. What the effective factor in the emulsion is, where the cholesterol goes, and what this means clinically, are, of course, unanswered questions of major interest.

Dr. Portman (15) has reported some studies at the Federation Meeting on the effects of different kinds of fat and different amounts of fat on the serum cholesterol levels of cebus monkeys receiving the atherogenic diets developed in our laboratories (16). These diets contain alpha protein, derived from soybeans, as a protein source, and 0.5% cholesterol. This amount of cholesterol is ten times less than originally used in such atherogenic diets, yet it produces the same degree of hypercholesteremia in the same length of time. Also, it is an amount of cholesterol which in terms of calories consumed is of the same order of magnitude present in typical American diets. With corn oil as the fat source, Dr. Portman found that raising the fat levels from 10 to 45% of the calories increased the serum cholesterol. However, the substitution of hydrogenated cottonseed oil, at either the high or low fat level, caused even greater serum cholesterol responses.

As a follow-up of this work, we selected a series of fats which differ rather widely in their fatty acid make-up and fed them to rats. The diets were based upon those worked out by our associate, Dr. Fillios, to produce atherosclerosis in the rat (unpublished data). They contained 20% of the fat under test. Eight fats were used in these studies ranging from coconut and butter fat as examples of saturated fats, to corn, sardine and tung oil as unsaturated fats. Serum cholesterol levels were determined at intervals, and the study is still underway. So far, the mean serum levels of cholesterol do not all seem to correlate with the saturation of the fat, although there is a tendency in that direction. For example, the rats receiving the saturated fats, coconut and butter, have cholesterol values of 597 and 548 mg. %. Those receiving cottonseed and corn oils have cholesterol values of 340 and 384 mg. %—values approximately 40% lower. However, feeding tung oil, a highly unsaturated oil, but one in which the unsaturation is conjugated, resulted in the highest cholesterol level of all, and hydrogenated cottonseed oil with half the degree of saturation of the non-hydrogenated oil gave essentially the same cholesterol level as the non-hydrogenated oil.

High fat diets, whether fed in formula form or in the usual way, are, of course, relatively low in carbohydrates. Other studies by Dr. Portman (17) have demonstrated in rats that the kind of dietary carbohydrate may be of some importance in affecting the disposition of cholesterol. It is known that the major metabolic products of cholesterol are the bile acids. By cannulation of

the bile duct, he was able to demonstrate a considerable difference in the bile acid output, depending upon the diet fed. Chow-fed rats excreted considerably more bile than animals receiving purified diets with sucrose as the carbohydrate source. This led to a study of various carbohydrates, with the demonstration that when starch is used in the diet, the bile acid output is larger than when various simple sugars are used. When cholesterol was included in the diet to produce a hypercholesterolemia, the starch-fed animals had lower serum levels of cholesterol.

Dietary proteins are involved in atherosclerosis, at least the experimental variety. The production of atherosclerosis in the monkey was accomplished in our laboratories by means of diets containing alpha protein, and prevented when casein was the dietary protein. This effect is presumably due to the low sulfur amino acid content, but this supposition has not been rigorously proven. Studies on other proteins are underway at the present time. Since atherosclerosis in man is associated with an abundant diet, it is difficult to believe that diets low in protein or low in essential amino acids can be a causative factor. However, the total protein intake as percentage of the calories does not vary greatly as is often assumed. Rather high protein intakes can be obtained on vegetable diets low in fat. For example in our Nigerian studies 93 grams of protein were calculated from a diet of 2,800 calories with only 8% of the calories coming from fat. The relative requirements of amino acids under such circumstances as well as possible interrelations between fat intakes and protein requirements are unexplored. In any event, these are implications that cannot be completely ignored.

Has there been a marked change in the American diet during the past one or two generations—a period in which the incidence of coronary heart disease and atherosclerosis has apparently increased? What are the principal changes?

Data of food consumption to estimate such changes are generally obtained by two methods: the official tables of food availability prepared by the Department of Agriculture, and survey data of families or particular groups. The first method, that of food availability also called food consumption data, consists of estimates of foodstuffs available for civilian consumption based on foods at the retail level. The data have not been adjusted for waste in homes or waste in institutions. Data on waste are usually not very reliable; however, quantity of waste is probably very important. For example, how much fat is discarded after the cooking of meats, bacon or fat frying? Have the cooking methods changed in this country so that more food is broiled and excess fat discarded? Since in periods of prosperity waste may be greater than in periods of austerity, food availability tables may not present a true picture of changes in the dietary.

From the data of the Department of Agriculture (18) it is found that from 1935 to 1955 the average caloric consumption per capita per day has decreased slightly, roughly 70 calories. Protein has increased from 90 to 97 grams, fat from 134 to 148 grams, and carbohydrates have decreased from 440 to 384 grams per day. The decreased use of potatoes and cereals and an increase in milk, meat and eggs seem to have been responsible for these changes in the food availability tables. Since consumption of cereals and potatoes has decreased, as well as total calories, the *percentage* of fat in the diet would show an increase even if the total fat consumed had not changed.

The second method of estimating food consumption is the dietary survey. Many studies ranging from approximately 1900 to the present day show a considerable variation in dietary intake depending mostly on the group and area under scrutiny. However, variations with time are not very striking. For example, a dietary study of college men in a 'boarding club' in 1891 showed 44% of the calories coming from fat (19). A survey of women eating at college clubs in 1894 reported 36% of the calories as coming from fat (20), and even the Harvard Crew of 1898 consumed a diet providing 39% of the calories as fat (20). A study done by our Department in 1953 of adult women showed the percentage of calories derived from fat ranged from 36 to 46% of the total (21). Thus, there may not have been an increase in fat consumption by Americans over the past 50 years as claimed by some.

It is, of course, true that the use of hydrogenated fats has increased. Thirty years ago these fats were relatively new products. However, the proportion of animal and vegetable fats in the diet has remained relatively constant. Again, quoting Department of Agriculture tables from 1935–1939, of 132 grams of fat available for human consumption 73% were from animal sources and 27% were vegetable fats (19). In the 1955 preliminary report (22), 148 grams of fat were available, 70% animal and 30% vegetable fat—very little change.

It may be of interest to mention that early margarines in this country were made partly of beef fat though for many years now hydrogenated vegetable fats have been used almost exclusively. Margarine in other countries is made of various types of fats, whale oil being used extensively in the United Kingdom (23). Interestingly, the per capita availability of lard is about the same now as it was in 1910 (19), but lard processing now includes refining, hydrogenation and decolorization—also in some cases addition of gum guaiac and various antioxidants (24).

It might be pointed out that an increase in the consumption of hydrogenated fats during the past 25 to 30 years does not necessarily mean a decrease in the intake of the essential fatty acids. This follows from a number of reasons:

1) While the use of hydrogenated vegetable oils has increased over the past several years so too has the use of these same oils in an unhydrogenated state and this latter use represents a substantial contribution of essential unsaturated fatty acids.

2) Gross measurements of changes of saturation induced via hydrogenation do not necessarily indicate the degree of change in the content of biologically active linoleic and linolenic acids. During hydrogenation the content of normal linoleate of an oil may be reduced to very small levels without a proportionate decrease in iodine number.

It should be obvious that much of what we hear these days about changes in our intake of fat—animal versus vegetable, saturated versus unsaturated—is not consistent with published facts.

In conclusion, of the many factors which must be considered in any discussion of atherosclerosis, some are certainly nutritional, and thus related to diet. The job of the experimentalist is to separate and evaluate the relative importance of the various factors involved. The progress that already has been made by the science of nutrition, in opening approaches to the study of atherosclerosis, provides a basis for hope that a major advance in this field may be at hand. In practical terms, this might mean that a rational therapeutic approach would become available and, of far greater importance, the possibility of preventing or delaying the onset of this prevalent and serious disease. Diets *can be* changed if we are sufficiently motivated, and if we know *how* they should be changed.

REFERENCES

1. Cowdry, E. V. (editor). *Arteriosclerosis: A Review of the Problem*. New York: Macmillan, 1933.
2. Brody, S. In: *Nutrition in Annual Review of Biochemistry*, edited by J. M. Luck. Stanford, California: Ann. Rev. of Biochemistry, Ltd., 1935.
3. Keys, A., J. Brozek, A. Henschel, O. Mickelsen and H. L. Taylor. *The Biology of Human Starvation*. Minneapolis: Univ. Minnesota Press, 1950.
4. Keys, A. *Voeding* 11: 539, 1952.
5. Gofman, J. W., B. J. Hardin, T. P. Lyon, F. Lindgren, B. Strisower, D. Colman and V. Herring. *Circulation* 5: 119, 1952.
6. Mann, G. V., J. A. Muñoz and N. S. Scrimshaw. *Am. J. Med.* XIX: 25, 1955.
7. Mann, G. V., B. M. Nicol and F. J. Stare. *Brit. M. J.* ii: 1008, 1955.
8. Edington, G. M. *Tr. Roy. Soc. Trop. Med. & Hyg.* 48: 419, 1954.
9. Higginson, J. and W. J. Pepler. *J. Clin. Invest.* XXXIII: 1366, 1954.
10. Kinsell, L. W., G. D. Michaels, J. W. Partridge, L. A. Boling, H. E. Balch and G. C. Cochrane. *J. Clin. Nutrition* 1: 224, 1953.
11. Ahrens, E. H., Jr., D. H. Blankenhorn and T. T. Tsaltas. *Proc. Soc. Exper. Biol. & Med.* 86: 872, 1954.
12. Beveridge, J. M. R., W. F. Connell, G. Mayer, J. B. Firstbrook and M. DeWolfe. *Circulation* 10: 593, 1954.
13. Hardinge, M. G. and F. J. Stare. *J. Clin. Nutrition* 2: 83, 1954.
14. Lever, W. F. and W. R. Waddell. *J. Invest. Dermat.* 25: 233, 1955.
15. Portman, O. W., F. J. Stare and D. Bruno. *Federation Proc.* 15: 1956.
16. Mann, G. V., S. B. Andrus, A. McNally and F. J. Stare. *J. Exper. Med.* 98: 195, 1953.
17. Portman, O. W., G. V. Mann and A. P. Wysocki. *Arch. Biochem.* 59: 224, 1955.
18. *Consumption of Food in the United States, 1909–1952*. Washington: U. S. Dept. of Agric., Handbook 62, 1953, p. 162.
19. Atwater, W. O. and C. D. Woods. *Studies of Dietaries*. Conn. Agric. Sta., 1891–1905, p. 174.
20. Atwater, W. O. *The Demands of the Body for Nourishment and Dietary Standards*. Storrs, Conn. Agric. Sta., Report, 1902–1903.
21. Beaudin, R. and J. Mayer. *J. Am. Dietet. A.* 29: 29, 1953.
22. *The National Food Situation*. Washington: U. S. Dept. of Agric., Agricultural Marketing Service, N.F.S. 74, 1955, p. 26.
23. Jacobs, M. *Foods and Food Products*, Vol. 1, New York: Interscience, 1944, p. 588.

INTER-SOCIETY SESSION

Symposium on Nutritional and Toxicological Studies on Irradiated Foods[1]

Chairman: T. E. FRIEDEMANN

INTRODUCTORY REMARKS

THIS SYMPOSIUM deals with a radically new process—the preservation of food with ionizing radiation.

Since this process does not depend upon high temperatures as does conventional sterilization, it is frequently referred to as 'cold sterilization.' Nevertheless, sufficiently high levels of ionizing radiation not only completely kill all forms of micro-organisms, but also inactivate the tissue enzymes present which are equally capable of causing food spoilage. Lower levels of radiation have successfully extended the shelf-life of pre-packaged fresh meat items for several weeks. Killing trichinae in pork, destroying insects in stored grain and cereal products, and eliminating sprouting during storage of potatoes and onions are other successful applications of non-sterilizing doses of radiation to the food industry.

There are many proposed sources of ionizing radiation to meet the anticipated requirements of the food industry. The use of high voltage x-ray machines is quite impractical. Radioactive cobalt, which is made by bombarding naturally occurring cobalt with neutrons in a reactor, requires considerable time to prepare and is available in only limited quantities. Recent technological advances in design of high-voltage generators makes beams of high-velocity electrons a practical source of radiation. But probably the most promising source is the highly radioactive fission products which represent the by-products of our rapidly increasing number of nuclear reactors. Little or no use is presently made of this tremendous source of gamma radiation.

Like all new processes, the sterilization of foods by radiation deserves careful study before it can be adopted commercially. We know that foods treated by the presently proposed methods do not become radioactive. The public must be convinced of this. It must be shown that the minute quantities of new compounds formed during irradiation are not toxic, and that the loss of nutrients is no greater than such losses occurring during thermal processing of foods.

Because of the military implications of this revolutionary process, the Armed Forces have sponsored an extensive investigation involving more than 40 universities and research installations in the United States. The program is under joint supervision of the Office of the Quartermaster General, whose interest is primarily in the technological and industrial developments; and of the Office of the Surgeon General, whose interest is in the toxicological and nutritional aspects of the process.

The criteria for evaluating the safety and nutritional adequacy has been established by the Surgeon General's Office (1) and by the Federal Food and Drug Administration (2). Chemical and physical tests must precede animal tests. Animal feeding screening tests will be followed by taste panel evaluations. Ultimately human feeding experiments must be conducted, since the most elaborate animal studies may not disclose

[1] Atlantic City, N.J., April 19, 1956. The views and conclusions that are contained in the papers from this symposium do not necessarily reflect those of the Department of Defense.

some significant factor harmful to man. All techniques at our disposal must be utilized to insure that any subtle changes produced by irradiation of food does not have a deleterious effect when these foods become available to large human populations.

The current research program was started in the spring of 1954 under the general direction of Colonel William D. Jackson and Dr. Ralph G. H. Siu of the Research and Development Division of the Office of the Quartermaster General; and Colonels Richard P. Mason and Tyron E. Huber of the Research and Development Division of the Office of the Surgeon General. Much of th technological advice and support has bee through the excellent efforts of Dr. Bruce H Morgan of the Quartermaster Food and Con tainer Institute in Chicago, and Dr. Herman F Kraybill of the Medical Nutrition Laboratory i Denver.

REFERENCES

1. *The Nutritional Adequacy of Foods Sterilized by Ionizing Radiation.* Staff Study, Army Med Nutrition Lab. June 1953.
2. LEHMAN, A. J. AND E. P. LAUG. *Nucleonics* 1 52, 1954.

EFFECT OF IRRADIATION STERILIZATION ON NUTRITIVE VALUE OF PROTEIN AND ENERGY OF FOOD[1]

B. Connor Johnson and V. Chalam Metta

From the Department of Animal Science, University of Illinois, Urbana, Illinois

Before any new food processing method such as irradiation sterilization can be put into general use, foods so processed must be proven satisfactory with respect to the effect of the processing on *a*) wholesomeness (lack of toxicity), *b*) acceptability (palatability) and *c*) nutritive value of both the micro- and the macronutrients. In the work at Illinois we have studied only the last of these, the effect of irradiation sterilization on the nutritive value of the macronutrients: protein and energy.

EXPERIMENTAL

Protein Studies. Considering first the effect of irradiation sterilization on protein, we have studied the animal proteins of beef and milk and the vegetable proteins of peas and lima beans. The effect of irradiation on the nutritive value of the proteins of these foods has been evaluated by the conventional methods of determination of digestibility and biological value (1). The foods were in all cases canned, frozen and irradiated frozen at 3 million r γ-ray irradiation at the Materials Testing Reactor, Arco, Idaho. After irradiation the foods were returned to us frozen and stored frozen along with non-processed controls. The foods studied were incorporated wet into diets, as shown in table 1 for the beef experiments, at a level to provide 10% protein (N $\times$ 6.25) on a moisture-free basis. Similar rations were used for the experiments in which lima beans, peas and milk served as protein source.

Table 2 gives the results for the experiments on beef. From these data it can be clearly seen that irradiation at 3 million r had no effect on either digestibility or biological value of beef protein.

In table 3 are given the data on peas. In this case and also for lima beans and milk, we have compared non-processed and heat-processed with irradiation-processed food. For peas and lima beans the heat processing consisted of cooking in the autoclave for 4 minutes at 15 lb. pressure. From the data in table 3 it can be seen that irradiation had no effect on the digestibility of the pea protein but that it did lower the biological value of the pea protein by approximately 8% as compared to non-processed or heat-processed peas.

In table 4 are given the data for lima beans. From this table it can be seen that irradiation processing does not significantly alter either the digestibility or the biological value of the protein of non-processed lima beans. On the other hand, it is clear from this table that heat processing markedly improves both the digestibility of protein (by approximately 10%) and also the biological value of the lima bean protein (by approximately 18%). These results would indicate that irradiation sterilization does not destroy the trypsin inhibitor present in lima beans and this is probably to be expected in view of the well-known resistance of enzymes to destruction by irradiation. Thus, irradiated lima beans are still essentially raw.

In table 5 is given a summary of all of our experiments on the effect of irradiation sterilization on milk. In the work on milk, evaporated milk which had not been sterilized was used as control and is termed raw milk. The biological value of 90 obtained for it is in good agreement with previous published results on entirely non-processed milk. The heat-sterilized evaporated milk was held at 242° for 14 minutes. As can be seen from the table, irradiation of this 'raw' evaporated milk at 3 million rep reduced the biological value of the milk protein. In order to elucidate the cause of this marked lowering in biological value due to irradiation, a series of experiments were carried out in which the irradiated milk was supplemented with various amino acids and the biological value determined. As can be seen from the table, lysine and histidine showed no supplementing effect when added to

[1] This paper reports research undertaken in cooperation with The Office of the Surgeon General under Contract DA-49-007-MD-544.

TABLE 1. COMPOSITION OF TEST DIETS

	Raw Beef Diet	Radiation-Sterilized Beef Diet	Standardizing (Low-N)
	gm %	*gm* %	*gm* %
Beef round (raw)	13.63*		
Beef round (radiation-sterilized)		13.59*	
Dried fat-free whole egg			5.40
Cerelose	10.00	10.00	10.00
Sucrose	10.00	10.00	10.00
Amidex	17.78	17.78	17.78
Starch	30.00	30.00	38.19
Lard	5.09	4.88	5.63
Mineral mix 446	4.00	4.00	4.00
NaCl	0.50	0.50	0.50
Vitaminized starch 691	5.00	5.00	5.00
Cod-liver oil	1.50	1.50	1.50
Wheat-germ oil	0.50	0.50	0.50
Woodflock	2.00	2.00	2.00
Total	100.0	100.0	100.0

* On moisture-free basis to supply 10% protein in the diet.

TABLE 2. DIGESTION AND NITROGEN METABOLISM DATA OF RAW AND RADIATION-STERILIZED BEEF

	Raw-Beef	Radiation-Sterilized
Apparent digestibility, %	91.8±0.08	92.3±0.50
True digestibility, %	100.0	100.0
Nitrogen balance (mg/gm N intake)	578.5±9.8	591.5±21.0
Biological value, %	78.0±1.0	78.6±1.5

TABLE 3. NUTRITIVE VALUE OF NON-PROCESSED, IRRADIATION-STERILIZED AND HEAT-PROCESSED SWEET PEAS (MEAN VALUES)

	Peas		
	Raw	Heat-Processed	Irradiation-Processed
True digestibility, %	92.5	92	91
Nitrogen balance (mg/gm N intake)	368	354	275
Biological value, %	59	58	50

irradiated milk. However, cystine was found to bring the biological value of irradiated milk to a level above that of the original non-processed milk. Since cystine is the limiting amino acid in milk, it would be expected that supplementation with cystine would increase the biological value. However, these data do show that no other amino

TABLE 4. NUTRITIVE VALUE OF NON-PROCESSED, RADIATION-STERILIZED AND HEAT-STERILIZED LIMA BEAN PROTEIN

(Mean Value)

	Lima Beans		
	Raw	Heat-Processed	Irradiation-Processed
True digestibility, %	73	85	75
Nitrigen balance (mg/gm N intake)	106	332	163
Biological value, %	50	68	51

TABLE 5. EFFECT OF AMINO ACID SUPPLEMENTATION OF IRRADIATION-STERILIZED MILK ON GROWTH AND NITROGEN METABOLISM OF THE RAT

	Gain/gm protein intake*	N Balance/gm N intake	Biological value of protein
	gm	*mg*	%
Raw milk	2.74	626	90
Radiation processed milk	2.50	506	74
Radiation processed milk and cystine	2.89	661	92
Radiation processed milk and lysine	2.30	510	73
Radiation processed milk and histidine	2.28	500	73

* A 10% protein level in the diets was used.

TABLE 6. RESULTS OF EXPERIMENT ON METABOLIZABLE ENERGY

	Non-Processed	Irradiation-Processed
Metabolizable Energy of Diets, %		
Diet 1	89.6	90.1
Diet 2	89.2	89.0
Diet 3	92.3	91.4
Metabolizable Energy of Nutrients, cal/gm		
Casein	4.56±0.298	4.51±0.220
Lard	8.82±0.314	8.87±0.391
Carbohydrate	3.87±0.196	3.78±0.299

acid was made unavailable (destroyed or bound) to the extent that it became limiting and it seems clear that the whole effect of irradiation on lowering the biological value of milk was due to its effect on cystine (destruction or binding).

Energy Studies. The effect of irradiation on the energy supplied by carbohydrate, protein and fat was studied by the method of Metta and Mitchell (2). In this method the metabolizable energy contributed by each of the three major

calorigenic nutrients is determined simultaneously. This is done by using three diets differing widely in the relative proportions of protein, carbohydrate and fat. The metabolizable energy of each of these three rations is determined and then factorized into values for the three energy-yielding nutrients using a multiple regression equation of the type: ME = cC + fF + pP, in which ME is the determined metabolizable energy for a diet while C, F and P are the amounts consumed in this diet of carbohydrate, fat and protein, respectively, and c, f and p are the constants determined by solving the three simultaneous equations and represent the metabolizable energy per gram of each of these nutrients. In all of the energy experiments synthetic rations were used which supplied casein as the protein source, lard as the fat source and a carbohydrate mixture consisting of sucrose, dextrin and starch (2:8:1). All of these rations were made up to contain 50% water because the principal effect of irradiation is on the ionization of water and the principal damage due to irradiation sterilization is caused by the free radicals formed as a result of ionization in the water phase. These experiments were carried out in two ways: in the first, the three diets were pre-mixed, canned, frozen and irradiated and stored frozen as in the previous protein experiments. The diets were then fed wet so that no other treatment effect was introduced into the experiment. In the second series of energy experiments the calorigenic diet ingredients were canned with water in the case of carbohydrate and protein and alone in the case of fat. They were then irradiation sterilized and the diets were mixed from these irradiated ingredients. Table 6 gives the results of the first of these series of experiments and shows clearly that irradiation processing had no effect on the metabolizable energy contributed by the protein, fat or carbohydrate of the ration. The results of the second experiment were entirely similar. Since it has been well established that irradiation will cause polymer formation in the case of fats and will cause similar damages in the case of protein and carbohydrate when the irradiation level is sufficiently high (3) it seems evident that there must be some irradiation level at which the energy value of a food nutrient could be depressed. However, from our present work, it appears that this irradiation level must be well above that required for sterilization.

SUMMARY

The irradiation sterilization of beef, beans, peas and milk has not been found to cause greater losses in the nutritive value of the protein of these foods than are caused by other food processing methods. Milk which is sensitive to heat has been found to be also sensitive to irradiation and the loss in protein value due to irradiation has been shown to be due to a loss (either by destruction or binding) in cystine. At a sterilization level of 3 million r irradiation caused no loss in the energy value of the carbohydrate, fat or protein of synthetic rations.

REFERENCES

1. Mitchell, H. H. *J. Biol. Chem.* 58: 873, 1924.
2. Metta, V. C. and H. H. Mitchell. *J. Nutrition* 52: 601, 1954.
3. Johnson, B. Connor. In: AAAS Symposium on *Atomic Energy and Agriculture*. C. C. Comar, editor (presented at Atlanta, Ga., meeting of AAAS, Dec., 1955). In press, 1956.

WHOLESOMENESS OF A GAMMA-IRRADIATED DIET FED TO CHICKENS[1]

C. H. Burns, L. E. Brownell and H. C. Eckstein

From the Fission Products Laboratory and the Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan

Ionizing radiation may be used within a few years for the preservation of food for human consumption. Before this is possible the absence of toxicity and the nutritional adequacy of such food must be established by suitable feeding studies. A long-term rat- or dog-feeding experiment involving a specific human food provides human ingesting it. The reliance on mammalian species as the only test animals might result in failure to notice possible deleterious changes. For this reason, a feeding and breeding experiment using chickens was included in the program supported by the Office of the Surgeon General, U. S. Army. The following is a report of this

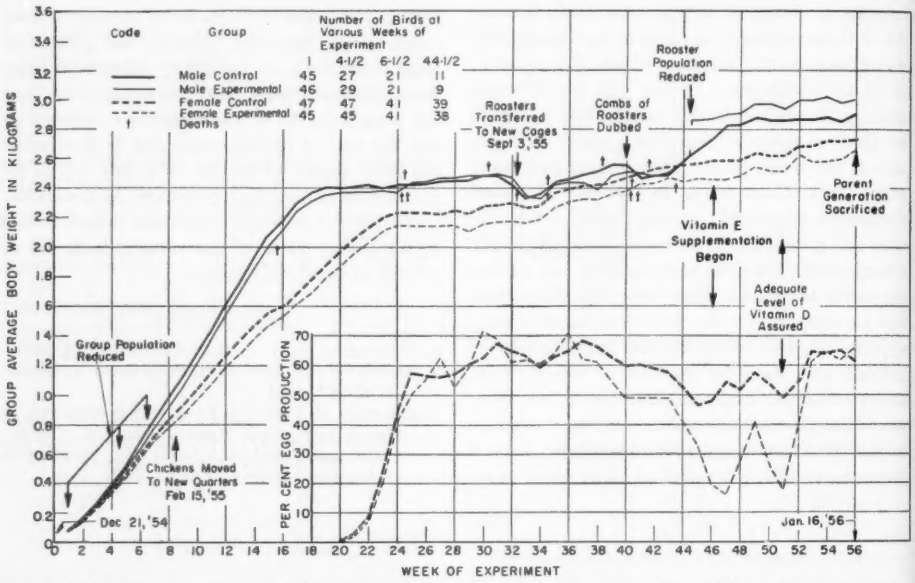


FIG. 1. Group mean body weights of the four groups of parent-generation chickens throughout the 56 weeks of experiment. Also shown on the same time scale is percentage egg production.

much of the evidence required by the Food and Drug Administration for clearance of the food, and a number of such experiments are in progress. Information is required on the nature of the chemical alterations occurring in irradiated food, and of the effect of such changes on the animal or experiment wherein chickens were raised and maintained for 13 months on a wet-mash diet which had received a sterilizing dose (three million roentgen equivalents physical) of gamma radiation.

The diet fed to the chickens consisted of nutritionally complete poultry mashes manufactured by the Ralston-Purina Co.: 'Startena' for chicks up to four weeks of age; 'Growena' for birds from four weeks of age to maturity and for

[1] This paper reports research undertaken in cooperation with the Office of the Surgeon General under Contract DA-49-007-MD-581.

roosters thereafter; 'Layena' for pullets after they reach 20% egg production, and a mixture of Layena and 'Booster Chow' when it is desired to hatch chicks from the eggs. These mashes contain the same ingredients, but the proportions vary to meet the different protein, calcium and phosphorus requirements of chicks, growing birds and breeding pullets. The ingredients include ground yellow corn, soybean oil meal, ground oats, wheat standard middlings, ground barley, ground grain sorghums, dehydrated alfalfa meal, corn gluten feed, meat scrap, fish meal, animal fat and a number of supplements to assure an adequate level of vitamins B-12, A and D, of thiamine and niacin, and of phosphate, calcium, salt and manganese. The basic food substances in these mashes are the same as those in a human diet, and many of the ingredients find their way into the human diet in other forms.

The mash was mixed with an amount of water equal to about 84% of its dry weight, because the free radicals formed in water contained in irradiated food are known to be responsible for many of the chemical alterations in foods. The mash was irradiated by exposing it to a 3000 curie cobalt-60 source of gamma radiation. To acquire the 3,000,000 rep dose, the period of irradiation was varied from 16 hours to 9 days depending on the position of the mash with respect to the source. The non-irradiated mash was not made up simultaneously because the period of storage at ambient temperature would allow spoilage and thus introduce a second variable.

It has been established in previous experiments that large dosages of ionizing radiation destructively alter many of the vitamins. Thus, to test

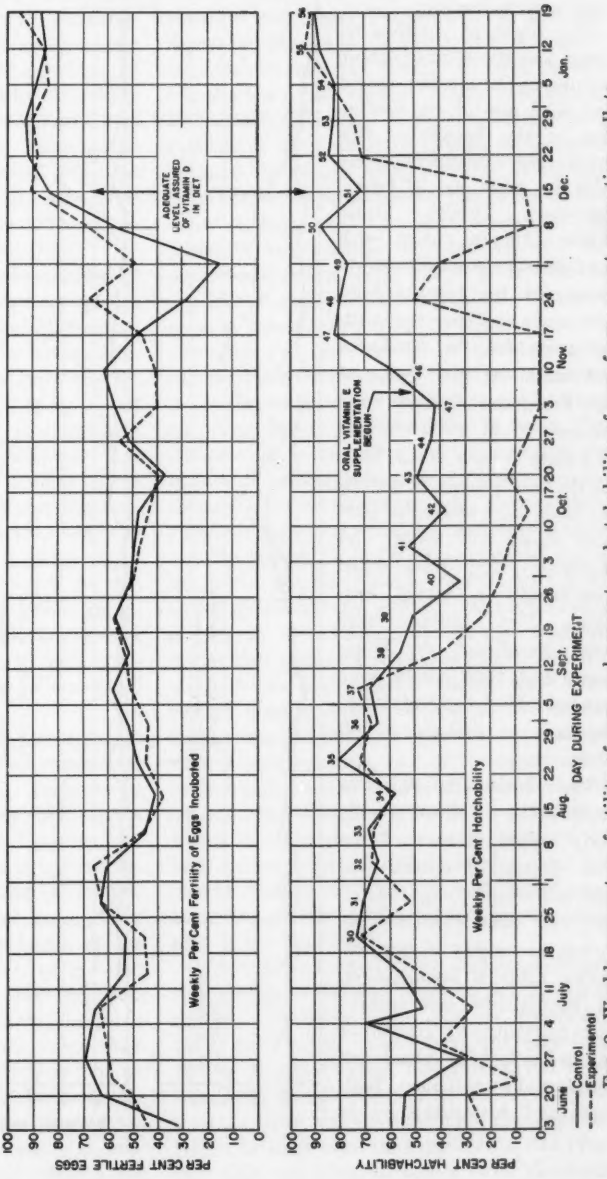


FIG. 2. Weekly percentage fertility of eggs and percentage hatchability of eggs from parent-generation pullets.

for the more subtle nutritional effects, and for chronic toxicity, a complete vitamin supplement was routinely mixed into the irradiated wetmash (and into the control diet as well). The wet mashes not stored in a deep-freeze were stored at 40°F not longer than three days prior to feeding.

The breed of chickens selected was the White Leghorn because of the medium weight and high rate of egg production of the females of this breed. The experiment began with 91 male and 92 female pure-bred Dryden strain White Leghorn chicks, each sex divided between the control group fed the non-irradiated wet mash and the experimental group fed the irradiated wet mash. At 6½ weeks of age, the group numbers were reduced to 21 cockerels and 41 pullets in each group. During the first 8 weeks of growth the birds were housed in pens with raised wire floors, and thereafter they were caged individually in laying batteries. The roosters were later transferred to larger cages and their combs were trimmed. Feed and water were supplied ad libitum to all birds.

The criteria followed in this study were *1*) mean weekly body weight of each of the four groups, *2*) efficiency of food utilization, *3*) egg production and percentage of hens laying, *4*) fertility of eggs following artificial insemination, *5*) hatchability and embryonic mortality, *6*) growth of chicks hatched, *7*) blood cell counts and *8*) pathology, both gross and histopathology of

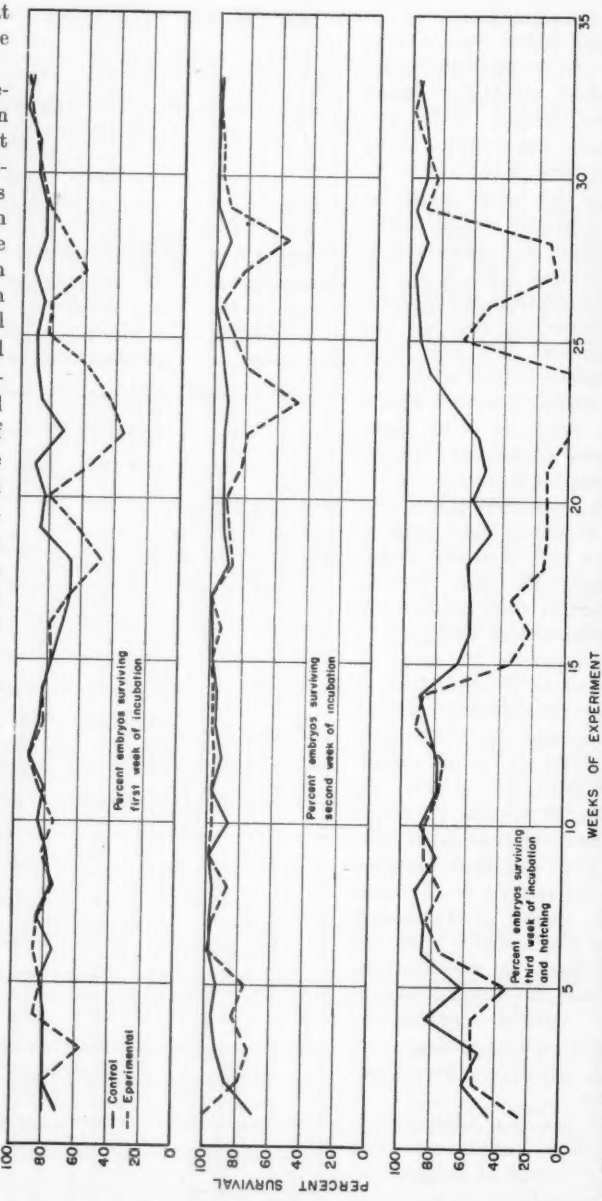


FIG. 3. Percentage survival of egg embryos from 1 week to the next during the 3-week incubation period in eggs from control and experimental pullets. The base of the percentage is the number of living egg embryos at the beginning of each of the 3 weekly periods.

tissues removed from birds dead or sacrificed.

Figure 1 shows plots of the mean weekly body weight of each of the four groups of chickens. Both experimental groups showed slight lags in growth rates, about 5% for the females and 3-4% for the males. The difference did not persist between the male groups after maturity. Beyond the 31st week, the data on the body weight for the male groups are not significant as a result of added variables caused by difficulties. The observed difference in body weight did persist and became slightly greater between the female groups. This finding is typical of experiments involving other species (rats and mice) fed diets of a very different character. The difference is always slight, but it is persistent.

The feeding of wet mash resulted in practically no waste, which is not the case when dry mash is fed. This allowed some measurement of efficiency of food utilization to be obtained by feeding both groups measured amounts of diet. From the 7th to the 13th week of experiment, 392 kg of wet mash (of constant water content, as determined by frequent dry-weight determinations) were fed to the control and the same amount to the experimental groups. The control groups gained a total of 46.1 kg during this period, while the experimental groups gained 43.7. This is an efficiency of food utilization of 94.7% for the experimental group relative to the control group. The equal consumption by both the control groups and experimental groups rules out acceptability as a factor in utilization of irradiated mash fed to chickens.

The data on egg production are also shown in figure 1. Until the 37th weeks of the experiment (or 17th week of egg production), there was no discernible difference in performance between the control and experimental pullets. A seasonal decline occurred after the 17th week of egg production, but the decline was more rapid and became precipitous for the experimental group. Oral vitamin E supplementation was begun at the indicated time in the belief that, as in other experiments, the peroxides and other oxidation products formed during irradiation exerted a destructive effect on the tocopherols, even though the latter were added after irradiation. The response to the oral vitamin E supplementation did not persist. A new source of cod liver oil was obtained and measures taken to assure an adequate level in the rations. This restored egg production to its previous level which indicated no apparent difference in rate between the pullets fed the irradiated and non-irradiated diet.

Production of eggs, fertile or infertile, by the pullet is, by itself, the evidence of the pullet's fertility. In order to obtain fertile eggs insemination of pullets was conducted artificially by a standard procedure. The fertility of the egg, shown for all eggs in the upper part of figure 2, requires not only the fertility of both parents but also the proper conduct of the insemination. Hence, using fertility of eggs produced as a measure of the potency or fertility of the male becomes meaningful only when a sufficiently large number of inseminations can be considered on a statistical basis to have minimized the effects of any artificial interferences with the opportunity of the sperm cell to unite with the egg. Eleven males on the non-irradiated diet were used to inseminate pullets on the control diet 109 times and those on the irradiated diet 106 times. Nine males on the irradiated diet were used to inseminate pullets on the control diet 64 times and those on the irradiated diet 78 times. The insemination of one pullet with the semen on one male was repeated after a week's interval, occasionally after two weeks' interval. The numerical value assigned to the result of the insemination was the number of fertile eggs produced during the period from the 2nd day after the 1st insemination to the 14th day after the 2nd insemination divided by the total number of eggs produced during that period. Expressed as percentages, the means for the four groups of matings were 47 for control male x control female, 49 for control male x experimental females, 59 for experimental male x control female, and 57 for experimental male x experimental female. Insemination with semen from experimental males resulted in a higher fertility score of the eggs produced by both groups of pullets than did that from the control roosters. However, the numbers of roosters involved were small and the difference probably is not significant.

Figure 2 shows plots of the weekly percentage hatchability of the eggs from the two groups of pullets. Up to the 32nd week of experiment (12th week of egg production), the eggs from control pullets hatched better than did those from the experimental pullets, but for the next 5 weeks there was no difference. The apparent failure to supplement the experimental diet properly with cod liver oil had a more disastrous effect on hatchability than it did on egg produc-

WEEKS OF EXPERIMENT

FIG. 3. Percentage survival of egg embryos from 1 week to the next during the 3-week incubation period in eggs from control and experimental pullets. The base of the percentage is the number of living egg embryos at the beginning of each of the

tion or number of pullets laying each week. Oral supplementation of vitamin E produced no permanent effect, but the assurance of an adequate level of a fresh source of cod liver oil brought an immediate response in hatchability. The higher levels of hatchability attained for both groups during the last few weeks of experiment was partly the result of improvements in the conditions of incubation.

Figure 3 shows the plots of the percentage of embryos during each of the 3 weeks of the normal incubation period. The percentages are based on the number of living embryos at the beginning of the corresponding weekly period. Differences in hatchability appear to be confined to the 3rd week of incubation, especially in the case of the situation corrected by proper cod liver oil supplementation. The high rate of embryonic mortality during the 3rd week is characteristic of a vitamin D deficiency in the maternal diet. A dosage of three megarep of gamma radiation apparently destructively altered the vitamin D originally present in the diet.

When male and female chicks hatched from pullets on each diet were raised on the irradiated and non-irradiated diet (to give eight combinations of sex, maternal diet and chick diet), the only observable distinction in rate of growth, other than in sex, was the diet of the chick. Whether the maternal diet was irradiated or non irradiated had no apparent effect on the rate of growth of the chick, if raised up to 28 days of age on either of the diets.

Table 1 presents the blood cell counts of representative animals from each of the four groups at the age of seven months. The counts were made by a technician with considerable experience in avian blood cell counting, and trained by Dr. Alfred Lucas of the U.S.D.A. Regional Poultry Research Laboratory. Considering the numbers of samples and the range of values observed, these data reveal no significant difference between the control and experimental animals with respect to blood cell populations.

During the 13 months of the experiment, 4 control and 6 experimental roosters out of 21 in each group died or were sacrificed because they were moribund, and 2 control and 3 experimental pullets out of 41 in each group died or were sacrificed. In nearly every case, sections of the vital organs were obtained at autopsy and submitted to histopathological examination The histopathology was carried out by Dr. Robert Hendrix and Dr. Ruth Wanstrom of the University of Michigan Department of Pathology. The pertinent observations recorded for the 15 birds are summarized in table 2.

Among the males, 2 in each group had distended gall bladders indicating an abnormality in the flow of bile. One in each group exhibited bronchitis, and another in each group showed acute passive congestion in the lungs, liver and kidneys. Of the remaining two experimental males, one exhibited extensive lymphocytic infiltrations in most of its tissues, and the last may have been afflicted with 'avian leukosis,' a dis-

TABLE 1. BLOOD-CELL COUNTS OF PARENT GENERATION CHICKENS ON LONG-TERM EXPERIMENT (June 1955)

Group	No. of Animals	Hematocrit	Hemaglobin in Whole Blood	Corpuscular Hemaglobin	Red Blood-Cell Count	White Blood-Cell Count	Polymorphonucleocytes	Differential Count % of White Blood-Cell Count				Average Thrombocyte Count
								Monocytes	Basophils	Eosinophils	Lymphocytes	
		%	%	%	*millions /cu mm*	*cu mm*						*Thousands/ cu mm*
Control Males	7	37.9 (30.6–41.0)	10.9 (9.8–11.9)	28.7	3.17 (2.74–3.44)	18,784 (9,523–31,818)	20.1 (8–33)	3.8 (0–8)	3.2 (0–6)	1.1 (0–4)	74.8 (59–88)	31.4 (14.6–57.1)
Experimental Males	7	36.4 (31.7–45.4)	10.7 (9.1–12.6)	29.4	3.09 (2.59–3.50)	22,439 (9,122–29,058)	15.6 (9–20)	2.9 (1–5)	1.9 (0–4)	0.86 (0–2)	78.6 (73–85)	31.8 (12.2–50.0)
Control Females	14	29.8 (25.4–71.6)	8.6 (7.6–9.9)	28.8	2.46 (2.07–3.03)	21,644 (11,986–39,878)	27.6 (16–44)	3.3 (0–10)	2.8 (0–8)	1.5 (0–5)	64.4 (45–80)	25.2 (9.7–41.0)
Experimental Females	14	28.3 (25.5–31.7)	8.3 (6.8–9.5)	29.3	2.53 (2.05–3.06)	24,800 (10,793–49,492)	25.1 (10–46)	1.9 (0–8)	2.7 (0–16)	0.8 (0–3)	69.2 (51–87)	27.1 (11.7–50.5)

TABLE 2. PATHOLOGY RECORD OF FIFTEEN PARENT-GENERATION CHICKENS LOST DURING 56 WEEKS OF EXPERIMENT

Animal No.	Order of Occurrence	Date Disorder Noted	Gross Observation	Date of Death or Sacrifice	Observations at Autopsy	Histopathological Observation
				Control Males		
05	3	25 Apr 55	Cannot support own weight	9 June (sac'd)		Heart: Occasional perivascular infiltration of lymphocytes; no lipoidosis Liver: No lipid in liver cells; fat droplets in some stellate cells of Kuppfer Spleen: Negative Pancreas: Negative Gizzard Negative Kidneys: Negative Small Intestine: negative Testis: Spermatogenesis active Lungs: Catarrhal bronchitis; bronchiectasis; no fat emboli
204	6	18 July 55	Rapid weight loss, refused diet	25 July (sac'd)	Enormously distended gall bladder; bile present in fore stomach and in feces	Gall Bladder: dilated Gizzard: Negative Heart: Negative Liver: No lipoidosis Kidneys: slight patchy degenerative fatty infiltration Testis: aspermatogenesis Spleen: negative Small intestine: negative Pancreas: negative Comment: dilatation of gall bladder
69	8	18 Aug 55	650-gram weight loss	18 Aug (died)	Autopsy showed greatly enlarged gall bladder and bile present in all parts of intestine.	(Tissues not submitted for histopathological examination because of post-mortem changes)
208	9	14 Sept 55	Sudden weight loss followed by death	14 Sept (died)		Heart: negative Lungs: congestion; hemorrhage by diapedesis; no lipid Spleen: negative Gizzard: negative Liver: acute passive congestion, lipid in lining cells of sinusoids Kidney: negative Testes: immature from mammalian criteria; no spermatozoa found; abundant lipid in interstitial cells
				Experimental Males		
167	1	4 Apr 55	Severe leg weakness, loss in weight	7 Apr (sac'd)		Spleen: congestion; erythropoietic foci Gizzard: appears normal Small intestine: negative Testis: spermatogenesis reduced Heart: numerous infiltrations of lymphocytes Liver: lymphocyte infiltrations in some of the trinities; no lipoidosis Pancreas: focal infiltrations of lymphocytes Lungs: congestion; patchy atelectasis; focal infiltrations of lymphocytes Kidney: focal infiltrations of lymphocytes; no lipoidosis Comment: It is not certain whether lymphocytic infiltrations are inflammatory or neoplastic; cells in vessels are the expected nucleated red blood cells
163	2	7 Apr 55	Head bent down, tends to squat, refuses food	9 June sac'd		Heart: negative Liver: negative Lungs: chronic catarrhal bronchitis Gizzard: negative Small intestine: Negative Kidneys: negative Spleen: negative Testis: spermatogenesis active

TABLE 2.—*Cont.*

Animal No.	Order of Occurrence	Date Disorder Noted	Gross Observation	Date of Death or Sacrifice	Observations at Autopsy	Histopathological Observation
				Experimental Males—Cont.		
157	4	27 Apr 55	Severe leg weakness, tends to collapse at ankles	9 June (sac'd)		Liver: slight degenerative fatty infiltration Heart: negative Kidney: no significant lipoidosis Spleen: vessels are thick-walled; numerous eosinophils Lungs: marked atelectasis; post-mortem change Gizzard: negative Testis: post-mortem change; spermatogenesis active Small intestine: negative
119	5	6 June 55	Neck twisted downward	6 June ? (died ?)	Enormously distended gall bladder and bile present in all parts of intestine	(Tissues not submitted for histopathological examination)
159	7	1 Aug 55	Died without prior weight loss	1 Aug (died)	Autopsy revealed green discoloration on right side, visible through skin, and also extending into muscle	Kidneys: congestion; no lipoidosis Liver: congestion Heart: negative Spleen: negative Testis: active spermatogenesis Gizzard: negative Adrenal and lung: no sections found either in fat stain or H and E
127	14	6 Oct 55	Comb excised on day of death; bird did not lose much blood, nor had it been losing weight	6 Oct (died)	No abnormality noted	Heart: negative Lung: acute passive congestion; slight hemorrhage; small amount of cellular exudate in bronchioles; acute passive congestion; no necrosis; very slight lipoidosis Kidney: no lipoidosis. Acute passive congestion Testes: active spermatogenesis
				Control Females		
84	12	29 Sept 55 (approx)	Died after week of disability, laid eggs to day of death	5 Oct (died)	Enlarged gall bladder, no egg formation beyond stage where yolk had diameter of ¾ inch; lung tissue appeared hemorrhagic	Heart: negative Lungs: acute passive congestion; much blood in respiratory antra but not in alveoli; no fat Liver: acute passive congestion; slight lipoidosis Kidney: acute passive congestion; similar to *female 138*; no lipid Note: there is one tissue here that cannot be identified (in addition to the four tissues listed, sections of the pancreas, oviduct, and duodenum were submitted for examination)
82	15	22 Jan 56	Died suddenly	22 Jan (died)	Profuse yellowish adhesions and yellow fluid, possibly ruptured oviduct; liver appeared hemorrhagic	Ovary: marked congestion Stomach: negative Small intestine: inflammatory infiltrations upon serosa; lipophages upon serosa Lungs: congestion, atelectasis Pancreas: negative Kidneys: congestion Heart: negative Spleen: marked congestion Gizzard: negative Liver: marked degenerative fatty infiltration Comment: peritonitis, as evidenced by reaction upon the peritoneum
				Experimental Females		
138	10	26 Sept 55	Died suddenly	26 Sept (died)	Large amount of depot fat, large liver, and portion of liver characterized by bloody infiltration and necrosis; oviduct full of eggs and appeared normal	Heart: small perivascular foci of mononuclear cells; no lipoidosis Lung: dilated respiratory passages; abundant mucin; peribronchiolar lymphocytic infiltration; definite pulmonary disease but classification will depend on study by an expert in poultry diseases Liver: definite and extensive necrosis of the liver; peripheral portions of lobule spared; much hemorrhage; abundant lipid in small droplets

TABLE 2.—*Cont.*

Animal No.	Order of Occurrence	Date Disorder Noted	Gross Observation	Date of Death or Sacrifice	Observations at Autopsy	Histopathological Observation
					Experimental Females—Cont.	
						Spleen: general architecture is not that of a mammalian spleen; lymphoid tissue diffuse; reticuloendothelial cells concentrated about blood vessels; germinal centers recognized in mammalian tissue are not seen—this may not be abnormal
						Kidney: differs somewhat from mammalian tissues; parietal and visceral epithelial cells of Bowman's capsule are conspicuous; I find no striking abnormality; no lipoidosis
218	11	26 Sept 55	Died suddenly, 2 days after laying an egg	26 Sept (died)	Extremely extensive malformation of all visceral connective tissue; a large cyst contained approximately 1 pint brown fluid; very numerous small sacs filled with blood clung to all surfaces	Heart: no lipoidosis
						Liver: abundant lipid in moderately large droplets; small foci of necrosis in random distribution; a few white and red blood cells are in these areas
						Gizzard: negative
						Intestine: severe active chronic inflammation involving serosal surfaces, mesentery, and subserosal tissues; at least part of this is in response to old hemorrhage, some amorphous material (mucin?) present, but there is no epithelial source for it
						Pancreas: negative
223	13	17 Oct 55	Died suddenly	17 Oct	Liver badly eroded; evidence of internal hemorrhage; oviduct contained large egg and other egg stages; large hard object in peritoneal cavity	Heart: negative
						Lungs: no abnormalities noted; no fat
						Liver: hemorrhagic necrosis; similar to but less extensive than female 138; marked lipoidosis
						Gall bladder: negative
						Intestine: negative
						Kidneys: similar to the others; no lipoidosis
						Note: An organ was found which cannot be identified; it is a cystic or tubular structure surrounded by adipose tissue; lining is columnar epithelium in low papillary folds; inside this there is a thin layer of calcium. Is this egg-shell production?

ease which frequently manifests itself in failure of muscular control.

Among the females, one in each group had severely abnormal visceral growths. The other control pullet had acute passive congestion in its tissues. The two other experimental pullets exhibited extensive liver necrosis. While the pathology of these birds is not sufficiently extensive to indicate the origin of the diseases, it is apparent that the occurrence of none of the abnormalities, except liver necrosis, was confined to birds in either dietary group.

The only observations having a bearing on the effect of irradiation of the diet were a slightly reduced rate of growth, and a slightly delayed maximization of hatchability of eggs from the pullets on the irradiated diet. All other observations made appeared to be negative with respect to the irradiation of the diet. It is possible that the slightly lower body weight may reflect mainly a difference in fat deposition of the experimental pullets, and that the delayed hatchability was a consequence of this.

It is concluded that no evidence indicating the presence of chronic or subacute toxicity or indicating gross nutritional losses other than vitamin losses was apparent in this experiment.

TOXICITY OF LIPID PEROXIDES IN THE RAT[1]

JOHN S. ANDREWS, JAMES F. MEAD AND WENDELL H. GRIFFITH

From the Department of Physiological Chemistry, University of California Medical Center, Los Angeles, California

SINCE it is well-known that fat is oxidized by irradiation, it follows that the toxicity of oxidized fat is an important part of the problem of food sterilization by this method.

In the investigations which are to be described it was assumed that the products of fat oxidation initiated by irradiation are essentially the same as the products of autoxidation. Moreover, the degree of oxidation was estimated by measuring the peroxide content of the fat. It should be noted, however, that there has been some lack of agreement on whether the principle toxic materials formed in overheated fats are the peroxides formed or the cyclic fatty acids which may also be present. In the present experiments it was found that if the peroxides present are not the toxic factor, then their concentration is closely correlated with that of the true toxic factor.

In the initial growth studies the following diet was fed to weanling rats: casein, 25%; sucrose, 46%; salts W, 4%; Brewer's yeast, 5%; lipid, 20%.

Soy bean oil was chosen as the lipid because of its extensive use, ready availability and high content of unsaturated fatty acids. Two kilograms of oil was oxidized at 60°C by a stream of air in the presence of 2 mg each of $FeCl_2$ and $CuCl_2$ until the peroxide number was 1200. The peroxide determination was carried out according to Polister and Mead (1) and the number refers to mEq of peroxide/kg of fat. The oxidized oil was stored at −20°C under nitrogen.

The oxidized oil was diluted with fresh to give oils with four different peroxide concentrations: 1200, 800, 400 and 100 PN. Four diets were mixed as previously described (containing one of the oxidized oils); a fifth diet containing fresh oil served as the control. Extraction of the lipids from these diets revealed that the peroxide numbers did not change on mixing or after 3 weeks storage at −20°C. Furthermore, the peroxide content of the diet did not change on standing for 3 days at room temperature. This observation is explained by the fact that the 1200 PN oil could not be further oxidized to any extent and that the lower peroxide number diets were obtained by dilution with raw oil. One hundred and seventy-five U.S.P. units of A, 35 units of D and 1.75 mg of α-tocopherol dissolved in 0.05 cc sesame oil were injected intramuscularly twice a week in order to satisfy the fat-soluble vitamin requirement.

After consuming the control diet for 6 days, five groups of 10 rats each were selected so that each group contained 5 males and 5 females. One of these groups was assigned to each one of the diets previously described.

Figure 1 presents the growth curves of the females over the first 70 days of the feeding experiment. The diet containing 20% fat with a peroxide number of 400 causes a reduction in growth, and increasing the peroxide number of the dietary fat increases the growth depression correspondingly until the 1200 PN diet is reached; consumption of this diet caused death within approximately 3 weeks. Similar effects on the growth of male rats fed these diets were observed. The only symptoms observed in rats consuming the 1200 PN diet were loss of weight and severe diarrhea. Animals receiving the 800 PN diet developed moderate to severe diarrhea, but this symptom subsided somewhat about the 8th week.

The question of palatability arises since this might account for differences in the food intake of the animals receiving the control, 400 and 800 PN diets. In order to test this hypothesis, the 'appetite quotient' as described by Carpenter (2) was calculated from the average food consumption and the average body weight of the animal fed each of the diets. The figures obtained indicate that sufficient food was consumed by each group to satisfy its growth requirements. Thus it would appear that the diets tested with a peroxide number of 400 or greater are toxic in

[1] This paper reports research undertaken in cooperation with the Office of the Surgeon General under Contract DA-49-007-MD-579.

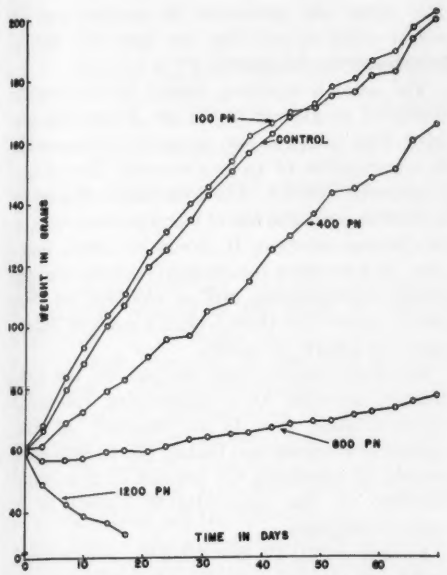


FIG. 1. Growth of female rats receiving various levels of oxidized fat.

TABLE 1. FERTILITY OF RATS RECEIVING DIETS CONTAINING PEROXIDE

Diet	No. Females	No. Pregnancies	Total Young	Total Reared
Control	4	2*	14	10
100 PN	5	5	23†	12‡
400 PN	5	5	33	21‡

* The two females which did not conceive weighed over 400 gm. † One litter born dead. ‡ One litter eaten.

TABLE 2. INTESTINAL XANTHINE OXIDASE ACTIVITY

Animal No.	Diet Consumed	Average % Transmission	% Control Activity
304	control	11	100
305		15	
315	100 PN	20	94
316		16	
322	400 PN	91	37
325		45	

some degree. The diet containing 20% fat with a PN of 100 is apparently non-toxic, according to the criterion of growth.

The animals raised and maintained on the control, 100 and 400 PN diets were bred at the age of 14 weeks. This experiment was unsuccessful, however, possibly because of a B vitamin deficiency. The results did indicate that oxidized fat had no effect on fertility.

After the Brewer's yeast had been enriched with B_{12}, folic acid and calcium pantothenate, and the yeast content of the diets increased to 7.5% at the expense of sucrose, the experiment was repeated. The only difference between this experiment and the previous one was that the males in this experiment were raised on a com-

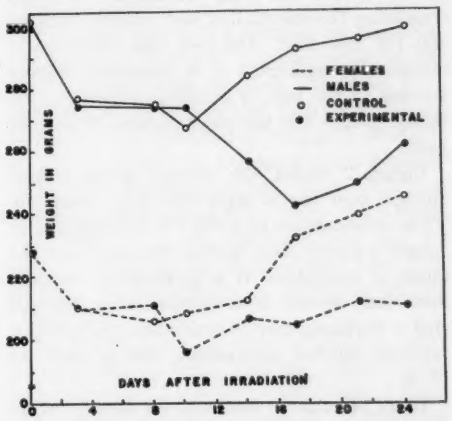


FIG. 2. Effect of diet on recovery from whole body irradiation.

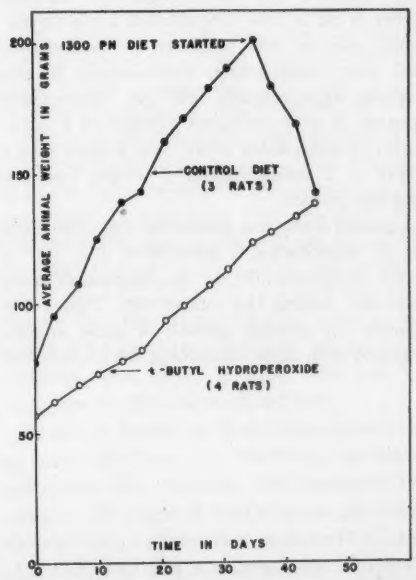


FIG 3. Growth of rats under several dietary regimes.

mercial diet. The results are given in table 1. It should be noted that the number of animals in each litter was reduced to six. The results of these limited experiments indicate that oxidized fat has no marked effect on either fertility or lactation.

After consuming the 100 PN diet for 8 weeks, 25 rats, 11 males and 14 females, along with concomitant controls were subjected to a whole-body x-irradiation dose of 600 r. During the 30-day period following irradiation, 3 males consuming the control diet and 7 males receiving 100 PN diet died. Although this difference is statistically significant it is somewhat suspect because of the lack of significance between the female groups and the small number of animals used.

Figure 2 shows the average group weight changes over the 27 days following irradiation. Thus, consumption of a 100 PN diet apparently caused a slower than normal recovery from the stress of irradiation. It is pertinent to mention here that several investigators have observed that a sterilizing dose of irradiation will induce a peroxide number approaching 100 in pork fat (3, 4).

In an attempt to ascertain the toxicity of an unequivocal peroxide, *t*-butyl hydroperoxide was synthesized according to the method of Milas and Surgenor (5). In preliminary injection studies, 8 mg of this compound in a non-physiological solution was found to be immediately fatal when administered intravenously to rats weighing approximately 400 gm. Intravenous injection of a physiological solution of 1 mg of *t*-butyl hydroperoxide every 3 or 4 days over a period of 2 weeks caused a weight loss and some loss of hair.

A growth study was conducted with 4 weanling rats by substituting a solution of 4×10^{-2} M *t*-butyl hydroperoxide for the animals drinking water and feeding the control diet. Figure 3 illustrates the average growth of these animals compared with those consuming the control diet. This figure also illustrates the marked loss of weight which occurs when the 1300 PN diet is substituted for the control.

The animals receiving *t*-butyl hydroperoxide consumed an average of 313 μM of peroxide per day. This amount of peroxide corresponds to consumption of an experimental diet of approximately 600 PN. The retardation of growth is obvious, and some loss of hair was observed but no diarrhea occurred. It should be noted, however, that no direct comparisons between feeding *t*-butyl hydroperoxide and an oxidized fat diet can be made since there is such a marked difference in solubility properties.

Intestinal esterase was assayed in the lyophilized intestine by a modification (personal communication from H. A. Ramsey) of the method of Harfenist and Baltzly (6). In essence it consists of measuring the amount of β napthol liberated by the enzymatic hydrolysis of 2 napthyl caprylate.

Feeding a 1300 PN diet for 2 weeks or *t*-butyl hydroperoxide for 6 weeks, as previously described, was found to have no effect on enzyme activity. Assay of xanthine oxidase in fresh intestinal tissue was conducted by means of a dye-coupling reaction employing blue tetrazolium. This investigation is still in the preliminary stages, but table 2 shows some limited data indicating a reduction in enzyme activity due to consumption of oxidized fat.

REFERENCES

1. B. H. Polister and J. F. Mead. Univ. of California at Los Angeles Report No. 253, 1953.
2. K. J. Carpenter. *J. Nutrition*. 51: 435, 1953.
3. A. L. Tappel, H. Groninger and F. W. Knapp. Report No. 5, Q.M. Food and Container Inst. for the Armed Forces, Chicago, 1955.
4. J. F. Mead and W. H. Griffith. Progress Report No. 3, Q.M. Food and Container Inst. for the Armed Forces, Chicago, 1955.
5. N. A. Milas and D. M. Surgenor. *J. Am. Chem. Soc.* 68: 205, 1946.
6. M. Harfenist and R. Baltzly. *J. Am. Chem. Soc.* 69: 362, 1947.

RADIATION EFFECTS ON WATER SOLUBLE VITAMINS IN RAW BEEF[1]

H. D. Alexander, E. J. Day, H. E. Sauberlich and W. D. Salmon

From the Department of Animal Husbandry and Nutrition, Agricultural Experiment Station, Alabama Polytechnic Institute, Auburn, Alabama

At the present time, very little information is available relating to the effect of ionizing irradiation on the water soluble vitamins in natural food substances. It has been reported that most of the synthetic vitamins in separate, dilute solutions are destroyed by exposure to irradiation, even at relatively low dosage levels. It has also been reported that some vitamins in their natural state are more resistant toward irradiation than are the synthetic forms.

We have made a study in which biological, microbiological and chemical methods have been used to determine the effect of gamma radiation on water soluble vitamins of raw ground beef. Beef was sealed in #2 tin cans, frozen and shipped in dry ice to Arco, Idaho, where it was given ca. 3,000,000 rep irradiation. Part of the same sample with identical treatment was shipped to serve as a nonirradiated control. A master control of the same sample was stored in a freezer in our laboratory. The basal diet used in the animal experiments contained 20% extracted casein, 5% fat, 4% salts and 71% sucrose. All vitamins except the one to be assayed for were added to the diet; ground beef at various levels supplied this vitamin. The casein and lard levels of the diet were adjusted at the expense of sucrose so that the protein and fat contents were constant throughout.

Data are presented in table 1*A* from an experiment in which growth of depleted weanling male rats was used to measure effects of irradiation on thiamine. For this assay it was necessary to add some thiamine to the test diet. At the 20% level of beef, results show that the irradiated beef contained 0.47 mcgm of thiamine per gm of fresh beef, whereas the nonirradiated meat contained 1.33 and 1.31 mcgm. The 30% level gave a value of 0.44 mcgm of thiamine per gm in the irradiated beef compared with 1.55 and 1.43 mcgm/gm in the nonirradiated beef.

In table 1*B* are data from a similar study to ascertain the effects of irradiation on riboflavin. In this study, depleted mixed-sex weanling rats were used, and some riboflavin was added to all diets. At the 5% level of beef, data show that the irradiated beef contained 2.7 mcgm of riboflavin per gm of fresh meat as compared with 2.8 mcgm/gm in the nonirradiated beef. When 30% beef was added to the basal diet a value of 2.4 mcgm was obtained for the irradiated beef and 2.9 and 2.7 mcgm for the nonirradiated beef. This indicates no destruction of riboflavin by the irradiation.

Results are given in table 1*C* from an assay for pyridoxine in which depleted, weanling male rats were used. At the 10% level of beef, a value of 2.2 mcgm of pyridoxine per gm of irradiated beef was obtained contrasted with 3.0 and 3.2 mcgm/gm in the nonirradiated beef. When 20% of beef was added, the values became 2.3 mcgm/gm of pyridoxine in the irradiated beef and 2.9 and 2.8 mcgm/gm in the nonirradiated beef. These values indicate that pyridoxine is more sensitive to irradiation than is riboflavin.

Data from microbiological and chemical determinations for thiamine made on three shipments of beef are given in table 2. Microbiological data show that the irradiated beef contained 0.52, 0.43 and 0.45 mcgm of thiamine per gm of fresh meat and that the nonirradiated beef contained 1.31, 1.32, 1.31, 1.35, 1.13 and 1.13 mcgm/gm. Results from the chemical method gave a value of 0.45 and 0.43 mcgm of thiamine per gm for the irradiated beef and 1.13, 1.13, 1.05 and 1.12 mcgm/gm for the nonirradiated beef.

In table 3, results are given from microbiological determinations for riboflavin, pyridoxine and niacin. The irradiated beef contained 1.65, 1.66 and 1.79 mcgm of riboflavin per gm of meat while the nonirradiated beef contained 1.87, 1.83, 1.85, 1.85, 1.82 and 2.16 mcgm/gm. Pyridoxine values of 1.70, 1.97 and 2.07 mcgm/gm were obtained for the irradiated meat compared with

[1] This work was conducted under Army Contract No. DA-49-007-MD-543 Surgeon General's Office.

2.32, 2.28, 2.55, 2.61, 2.81 and 2.83 mcgm/gm for the nonirradiated beef. The niacin content was shown to be 40.0, 43.6 and 48.1 mcgm/gm in the irradiated beef compared with 40.8, 42.0, 40.1, 39.7, 50.7 and 46.9 mcgm/gm for the nonirradiated beef. Some destruction of riboflavin and pyridoxine by the irradiation was noted here. Niacin, however, was resistant toward the irradiation.

Results from determinations for choline and folacin are given in table 4. Choline was measured by the Reineckate method and folacin by microbiological assay. The irradiated beef contained 860 mcgm of choline chloride equivalent per gm of fresh meat as compared with 830 and 843 mcgm/gm for the nonirradiated. The folacin content was shown to be 0.027 mcgm/gm in the

TABLE 1. ASSAY OF THIAMINE RIBOFLAVIN AND PYRIDOXINE IN BEEF AS MEASURED BY GROWTH OF DEPLETED WEANLING RATS (28-day assay period)

Beef plus Basal Diet	Av. food/ rat/day gm	Av. wt. gain/rat gm	Vitamin derived from beef (wet basis) mcgm/gm
*A. Thiamine**			
20% Irradiated	11.0	92	0.47
20% Nonirradiated	13.4	127	1.33
20% Nonirradiated MC†	15.3	146	1.31
30% Irradiated	13.0	113	0.44
30% Nonirradiated	18.9	186	1.55
30% Nonirradiated MC†	19.7	187	1.43
B. Riboflavin‡			
5% Irradiated	6.8	30	2.7
5% Nonirradiated	7.0	31	2.8
5% Nonirradiated MC†	6.7	31	2.8
20% Irradiated	9.7	57	2.4
20% Nonirradiated	10.3	64	2.9
20% Nonirradiated MC†	9.4	60	2.7
C. Pyridoxine			
10% Irradiated	12.6	70	2.2
10% Nonirradiated	13.7	88	3.0
10% Nonirradiated MC†	13.6	91	3.2
20% Irradiated	17.3	124	2.3
20% Nonirradiated	17.8	140	2.9
20% Nonirradiated MC†	19.5	144	2.8

* Thiamine added at a level of 750 mcgm/kg of diet in all groups.
† Master control.
‡ Riboflavin added at a level of 250 mcgm/kg of diet in all groups.

TABLE 2. MICROBIOLOGICAL AND CHEMICAL ASSAY OF THIAMINE IN IRRADIATED AND NONIRRADIATED BEEF*

Treatment of Beef	Method of Assay: Microbiological (wet basis) mcgm/gm	Chemical (wet basis) mcgm/gm
Irradiated	0.52	0.45
Nonirradiated	1.31	1.13
Nonirradiated MC†	1.32	1.13
Irradiated	0.43	0.43
Nonirradiated	1.31	1.05
Nonirradiated MC†	1.35	1.12
Irradiated	0.45	
Nonirradiated	1.13	
Nonirradiated MC†	1.13	

* Determination made on 3 shipments of beef.
† Master control.

TABLE 3. MICROBIOLOGICAL ASSAY OF RIBOFLAVIN, PYRODOXINE AND NIACIN IN IRRADIATED AND NONIRRADIATED BEEF*

Treatment of Beef	Vitamin Assayed: Riboflavin (wet basis) mcgm/gm	Pyridoxine (wet basis) mcgm/gm	Niacin (wet basis) mcgm/gm
Irradiated	1.65	1.70	40.0
Nonirradiated	1.87	2.32	40.8
Nonirradiated MC†	1.83	2.28	42.0
Irradiated	1.66	1.97	43.6
Nonirradiated	1.85	2.55	40.1
Nonirradiated MC†	1.85	2.61	39.7
Irradiated	1.79	2.07	48.1
Nonirradiated	1.82	2.81	50.7
Nonirradiated MC†	2.16	2.83	46.9

* Determination made on 3 shipments of beef.
† Master control.

TABLE 4. CHOLINE AND FOLACIN IN IRRADIATED AND NONIRRADIATED BEEF

Treatment of Beef	Nutrient Assayed: Choline Cl (wet basis) mcgm/gm	Folacin (wet basis) mcgm/gm
Irradiated	860	0.027
Nonirradiated	830	0.024
Nonirradiated MC*	843	0.025

* Master control.

TABLE 5. SUMMARY OF IRRADIATION EFFECTS ON CERTAIN WATER SOLUBLE VITAMINS IN BEEF

Vitamin Assayed	Percentage Destruction Determined by: Animal	Microbiological	Chemical
Thiamine	67	63	60
Riboflavin	8	10	
Pyridoxine	24	25	
Niacin		None	
Choline			None
Folacin		None	

irradiated beef and 0.024 and 0.025 mcgm/gm in the nonirradiated meat. Both choline and folacin were resistant toward the irradiation.

A summary of the effects of irradiation on water soluble vitamins is given in table 5. Values for the vitamins obtained by biological, microbiological or chemical methods compare favorably with each other. Thiamine was significantly decreased: 67% as revealed by animals, 63% by microbiological and 60% by chemical method. Some riboflavin was destroyed: 8% as revealed by animal assay and 10% by microbiological assay. Pyridoxine destruction was calculated to be 24% when measured by biological test and 25% when measured by microbiological assay. Niacin, choline and folacin were not detectably decreased by the ionizing irradiation treatment used.

EFFECT OF IONIZING RADIATIONS ON VITAMIN K[1]

LUTHER R. RICHARDSON, PATRICIA WOODWORTH AND SANDRA COLEMAN

From the Department of Biochemistry and Nutrition, Texas Agricultural Experiment Station College Station, Texas

THIS STUDY is one phase of the overall investigation on the radiation preservation of foods, which is being carried out by the Quartermaster Corps and the Office of the Surgeon General. One phase of the investigation carried out in this laboratory was the effect of ionizing radiations on the diet of some of the micronutrients. The results of studies on the effect of ionizing radiations on vitamin K, from different sources, are described in this report.

EXPERIMENTAL

Chicks were used for the bioassay of the vitamin K activity of the foods containing different sources of vitamin K. The sources of vitamin K used were menadione (K^3), 3-phytylmenadione (K_1), 2-methyl-4-amino-1-naphthol HCl (K_5), 3-dehydrated alfalfa leaf meal and fresh spinach. Day-old White Leghorn cockerels were kept in electrically heated batteries and feed and water were supplied ad libitum. There were 20 chicks per group and the experimental period was 18 days. Precautions taken to minimize the possibility of the chicks obtaining vitamin K from bacterial synthesis were: *1*) left-over feed was discarded and the feed troughs were cleaned thoroughly twice weekly; *2*) water troughs were cleaned daily and 0.15% of sodium benzoate was added to the drinking water.

Basal Diet. The composition of the synthetic basal diet is given in table 1. The diet contained all of the macronutrients and vitamins, with the exception of vitamin K, known to be required for a rapid rate of growth in chicks. The deficient diet, and the control diet which contained 50–100 mcg of menadione per 100 gm of diet, were mixed in 3 kg lots as needed.

Irradiated and Untreated Diets. The irradiated and untreated diets were shipped to the Materials Testing Reactor at Idaho Falls, Idaho, for irradiation. The diets were sealed in #2 plain tin cans, frozen, packed in dry ice in insulated containers and shipped to the irradiation service by way of Railway Express. One portion of the diet was irradiated at a dose level of 3×10^6 rep and an irradiation temperature of 72°F. This was the irradiated material. The other portion was treated the same way, with the exception of irradiation. This was the untreated material. The irradiated and untreated materials were then packed in dry ice in insulated containers and returned to the laboratory by way of Railway Express. The diets were stored in the laboratory in the frozen condition until they were used.

TABLE 1. COMPOSITION OF BASAL DIET

Constituents	Amt.	Vitamins/100 gm. of diet	Amt.
	gm		
Casein (vitamin free)	25	Vitamin A	3000 IU
Gelatin	8	Vitamin D	400 IU
Cerelose	48.6	a-Tocopherol	2.0 mg
Woodpulp	3	Thiamine HCl	1.0 "
Mineral mixture*	5	Riboflavin	1.0 "
Lard	10	Pyridoxine HCl	1.0 "
Choline chloride	0.3	Ca pantothenate	3.0 "
Methionine	0.1	Niacin	2.0 "
Inositol	0.01	Folic acid	0.2 "
		Biotin	0.02"
		Vitamin B_{12}	1.0 mcg

* Richardson, L. R. and A. G. Hogan. *J. Nutrition* 32: 459, 1946.

Vitamins A, D and E were added to both the irradiated and untreated diets at the beginning of each feeding test or whenever a new lot of diet was needed. These supplements were in addition to the vitamin A, D and E which were added to the diet before it was shipped to the irradiation center. In some tests, each chick received a supplement of vitamin A, D and E twice weekly. In this case, the vitamins were dissolved in Mazola and given by medicine dropper. All sources of vitamin K, with the exception of fresh spinach, were mixed into the diet before it was irradiated. The fresh spinach was irradiated by the same procedure as that described for the diets and mixed with the other constituents of the diet which were not irradiated.

Blood-Clotting Time. Blood-clotting time was determined by the same method as that used by many medical technicians. Briefly, it was as follows: a wing vein was punctured with a lancet and 5–10 drops of blood were collected on a

[1] This paper reports research undertaken in cooperation with the Office of the Surgeon General under Contract DA-49-007-MD-582.

TABLE 2. INCIDENCE OF HEMORRHAGE AND AVERAGE BLOOD CLOTTING TIME OF CHICKS RECEIVING IRRADIATED, UNTREATED AND CONTROL DIETS CONTAINING DIFFERENT AMOUNTS OF MENADIONE

Series	Menadione kg/of diet	Irradiated					Untreated					Control				
		No. of trials	Total chicks obs.	Hemorrhagic			No. of trials	Total chicks obs.	Hemorrhagic			No. of trials	Total chicks obs.	Hemorrhagic		
						Clot. time*					Clot. time*					Clot. time*
	mcg		*no.*	*no.*	*%*	*min.*		*no.*	*no.*	*%*	*min.*		*no.*	*no.*	*%*	*min.*
A	None											9	181	126	70	21
B	50	4	74	70	95	27	5	89	35	39	23	1	20	4	20	11
C	100	3	56	48	86	25	1	20	9	40	12	2	39	2	5	12
D	300	2	40	36	90	23	1	20	None	None	None					
E	500	2	40	12	30	19										
F	1000	3	60	21	35	17										
G	2000	2	39	6	15	17										

* Clotting time = average of blood which clotted in 10–30 min.

TABLE 3. INCIDENCE OF HEMORRHAGE AND AVERAGE BLOOD-CLOTTING TIME OF CHICKS RECEIVING IRRADIATED AND UNTREATED DIETS CONTAINING DIFFERENT SOURCES OF VITAMIN K_1

Amt. of supplement added	Irradiated					Untreated				
	No. of trials	Total chicks obs.	Hemorrhagic			No. of trials	Total chicks obs.	Hemorrhagic		
					Clot.* time					Clot.* time
		no.	*no.*	*%*	*min.*		*no.*	*no.*	*%*	*min.*
Series H										
Vitamin K_1 (3-phytylmenadione) mcg/kg										
333	2	40	4	10	13	3	58	2	3	11
1665	2	36	1	3	4					
3333	1	20	None	None	None					
Series I										
Vitamin K_5 (2-methyl-4-amino-1-naphthol) mcg/kg										
333	3	58	23	44	22	3	60	None	None	None
1665	2	37	None	None	None					
3333	1	20	None	None	None					
Series J										
Dehydrated Alfalfa Leal Meal %										
.25	1	19	3	16	12	1	20	None	None	None
.5	2	40	24	60	19	2	40	6	15	12
1.0	1	20	None	None	None	1	20	8	40	17
2.5	1	20	1	5	20					
Series K										
Fresh Spinach %										
1.0	2	40	11	27	16	2	40	17	42	18
2.0	1	20	2	10	10	1	20	4	20	21

* Clotting time = average of blood which clotted in 10–30 min.

clean microscope slide. A hypodermic needle with the point bent was drawn through the blood at one-half minute intervals. The appearance of fibrin on the point was taken as the clotting time. Chicks were considered normal if the blood clotted in less than 10 minutes and hemorrhagic if it clotted in 10 minutes or more. No clotting time over 30 minutes was recorded. The clotting time for the hemorrhagic chicks in the tables is the average of all chicks whose blood clotted between 10 and 30 minutes.

RESULTS AND DISCUSSION

Irradiated Diets Containing Menadione. The incidence of hemorrhage and the average blood-clotting time of chicks receiving the irradiated, untreated and control diets which contained different amounts of menadione are summarized in table 2. A comparison of the irradiated, untreated and control diets which contained 0, 50, 100 and 300 mcg of menadione per kg of diet is shown in *series A, B, C* and *D*. The incidence of hemorrhage in chicks (*series B, C* and *D*) receiving the irradiated diet ranged from 86 to 95% and the

average blood-clotting time ranged from 23 to 27 minutes. When the chicks received the control diet (*series A*) without menadione, 70% had an average blood-clotting time of 21 minutes. When the control diet (*series B*) contained 50 mcg of menadione per kg, 20% had an average clotting time of 20 minutes and when it (*series C*) contained 100 mcg, 5% had an average clotting time of 12 minutes. When it (*series D*) contained 300 mcg, the clotting time of every chick was below 10 minutes.

The incidence of hemorrhage in groups (*series B* and *C*) receiving the untreated diet was higher than in the control groups receiving equivalent amounts of menadione. These data show that some vitamin K was lost during shipment and storage of the diet, but the loss was much less than in the irradiated diets.

Similar trials with irradiated diets containing 500, 1000 and 2000 mcg of menadione per kg are shown in *series E*, *F* and *G*, respectively. There was some variation in the results with diets irradiated at different times but, with two exceptions, the incidence of hemorrhage was relatively low when the diet contained a large amount of menadione. In one group receiving 500 mcg of menadione, 45% of the chicks were hemorrhagic and in another group receiving 1000 mcg, 75% were hemorrhagic.

Irradiation of Vitamins K_1, K_5, Dehydrated Alfalfa Leaf Meal and Fresh Spinach. Vitamin K occurs in natural foods in forms other than menadione and it was desirable to determine whether the vitamin was destroyed by the irradiation process when different sources were used. Two pure compounds, vitamins K_1 and K_5, and two natural sources, dehydrated alfalfa leaf meal and fresh spinach, were tested. The results with these materials are summarized in table 3. The amounts of vitamin K_1 and K_5 shown in the table are equivalent in activity to approximately 100, 500 and 1000 mcg of menadione. The incidence of hemorrhage was low and the blood-clotting time was relatively short at each level of vitamin K_1 (*series H*) tested. These data indicated that practically none of the vitamin K activity was lost by the irradiation process when vitamin K_1 was the source of the vitamin in the diet.

With a low level of vitamin K_5 (333 mcg, *series I*) in the diet, the effect of irradiation was inconsistent. In one trial, 44% of the chicks was hemorrhagic. In another, 75% was hemorrhagic and in a third none was hemorrhagic. None of the chicks receiving the untreated diet was hemorrhagic. The irradiated diet containing large amounts of vitamin K_5 had sufficient vitamin K activity to prevent hemorrhage in any of the chicks. These data show that menadione is more readily destroyed by ionizing radiations than either vitamin K_1 or K_5.

Dehydrated alfalfa leaf meal and fresh spinach are rich natural sources of vitamin K activity. These two materials were tested in the irradiated studies to determine the effect of irradiation on the vitamin K activity when a natural source of the vitamin was added to the diet. Dehydrated alfalfa leaf meal was mixed in the diet and then the entire diet was irradiated. Some destruction of the vitamin by irradiation was evident when the alfalfa leaf meal (*series J*) was supplied in the diet at a level of 0.25 and 0.5%. When the level was 1 or 2.5%, only 1 chick out of 40 had a blood-clotting time longer than 10 minutes. This is not interpreted as an indication of a vitamin K deficiency. With relatively large amounts of dehydrated alfalfa leaf meal in the diet, there is still sufficient vitamin K activity left after irradiation to prevent hemorrhage.

As pointed out previously, fresh spinach was irradiated, then mixed in the diet which contained non-irradiated ingredients. Since the incidence of hemorrhage was higher in the chicks receiving the untreated spinach (*series K*) than it was in those receiving the irradiated spinach, it was concluded that no destruction of vitamin K occurred by the irradiation process.

SUMMARY

Menadione was more readily destroyed by ionizing radiations than either vitamins K_1 or K_5. When relatively large amounts of the three compounds were added to the diet, sufficient vitamin K activity was left after irradiation to prevent hemorrhage. Practically no destruction of vitamin K occurred when a diet containing dehydrated alfalfa leaf meal was irradiated or when fresh spinach was irradiated. It is difficult to produce a vitamin K deficiency by normal dietary means because of bacterial synthesis of the vitamin in the intestine. Furthermore, the loss of vitamin K activity was comparatively small when a diet containing a natural source of the vitamin was irradiated or when a natural food was irradiated. Therefore, it is concluded that no animal, including humans, receiving irradiated diets composed of natural foodstuffs, would ever develop a hemorrhagic condition because of a deficiency of vitamin K in the diet.

Acknowledgment is made to Merck & Co., Rahway, N. J., for generous supplies of vitamins and to Lederle Laboratories, Division of American Cyanamid Co., Pearl River, New York, for folic acid.

WHOLESOMENESS AND POSSIBLE CARCINOGENICITY OF RADIATED FOODS[1]

L. J. Teply and B. E. Kline

From the Wisconsin Alumni Research Foundation, Madison, Wisconsin

To provide certain preliminary data required by the Office of the Surgeon General, Department of the Army, 24 food items (control, and radiated at 3×10^6 and 6×10^6 rep) were subjected to short-term rat feeding tests according to procedures developed by Read, Kraybill and Witt (1) at the Medical Nutrition Laboratory, Fitzsimons Army Hospital, Denver, Colorado.

In brief, the control and radiated foods were fed for 8 weeks to 10 male rats per group. The foods were mixed with a complete semi-synthetic basal ration at a 35% level on a dry weight basis. Food consumption records were kept and feed efficiencies calculated. The items tested were: apricots, asparagus, Brussels sprouts, cabbage, cauliflower, celery, cherries, chicken, corned beef, cranberries, crackers, gelatin dessert powder, macaroni, mushrooms, nut roll, pears, peas, pork sausage, potatoes (sweet), potatoes (white), pound cake, salmon, shrimp and tuna. In the case of white potatoes, an extra dose level of 25,000 rep was included because this is in the range of dosages used to prevent sprouting. Perishable items were radiated and stored in the frozen state; test diets containing these items were mixed frequently and were also stored in the frozen state.

Growth results were analyzed statistically using weight gain and final weight as covariants and in this report the 0.05 level of probability is regarded as the dividing line for significance. In most cases there was no significant difference in growth on control and radiated foods. Radiation of cauliflower, celery and white potatoes produced a moderate increase in growth rate. These items were treated in the raw state and the growth effect may be due to improved acceptability or digestibility. A slight decrease in growth rate was produced by radiation of apricots and chicken, which are being subjected to repeat tests. Growth on all the cranberry diets was somewhat erratic and this product is also being subjected to additional experiments.

Radiation of gelatin dessert powder caused a definite and repeatable lowering of growth rate of approximately 10%, with a comparable decrease in feed efficiency. Microbiological assays showed destruction of several amino acids through radiation (table 1). However, feeding studies with sucrose and gelatin fed separately indicated that the sucrose component was responsible for most, if not all, of the growth effect. Gelatin dessert powder contains about 90% sucrose and 10% gelatin. The results in table 2 show that if these components are fed in this proportion, the growth effect is the same whether the components are radiated separately or as a mixture. In an attempt to accentuate the effect, all of the starch (52%) in the basal was replaced with sucrose and the gelatin was fed at an 8% level, with the casein reduced from 20 to 12%. Again a definite reduction in growth rate was obtained on radiation of sucrose while little effect was apparent on radiation of gelatin. The mechanism of the sucrose effect has not been determined.

The dessert powder, sucrose and gelatin were air dried and treated with beta radiation at room temperature. They were packaged for treatment in polyethylene bags. Radiation produced a slight yellowing of the sucrose.

Richardson (2) observed definite lowering of nutritive value of gelatin for the chick on radiation and also reported indication of destruction of arginine and glycine. The amino acid requirements of the chick are somewhat different than those of the rat. The state of the gelatin during radiation, (e.g. mixture of the air-dried gelatin with sucrose) may affect the pattern of amino acid destruction, or microbiological amino acid assays on a radiated gelatin product may not reflect exactly nutritive value for animals.

[1] The work reported herein was conducted under Dept. of the Army, Office of the Surgeon General, Medical Research Contracts Nos. DA-49-007-MD-583 and DA-49-007-MD-608

TABLE 1. AMINO ACIDS IN GELATIN DESSERT POWDER

	mg/gm		
	Control	3×10^6 rep	6×10^6 rep
Arginine	9.6	9.4	8.4
Cystine	0.12	0.09	0.09
Glycine	29.9	29.5	30.2
Histidine	0.7	0.7	0.4
Isoleucine	2.6	2.6	2.3
Leucine	2.5	2.5	2.4
Lysine	7.3	6.5	6.4
Methionine	0.98	0.81	0.80
Phenylalanine	2.3	2.1	1.8
Threonine	3.0	3.7	2.6
Valine	4.1	3.6	3.3

TABLE 2. RAT GROWTH ON RADIATED SUCROSE AND GELATIN

Diet	Avg. Wkly Gain, Gm (8 weeks)
35% Sucrose-gelatin mixture	34.0
35% Radiated sucrose-gelatin	30.5
35% Radiated sucrose and gelatin (radiated separately)	30.5
52% Sucrose	36.0
52% Radiated sucrose	32.0
8% Gelatin, 12% casein	30.0
8% Radiated gelatin, 12% casein	28.5

TESTS FOR POSSIBLE CARCINOGENICITY

A number of reports have suggested that overheating of foods, particularly fats, or oxidation of sterols (3) may result in the production of compounds that are carcinogenic to test animals. The exact significance of such findings in terms of human nutrition is difficult to assess, especially since a natural food component like cholesterol itself has been reported to be a mild carcinogen (4). The use of certain cooking methods, or addition of chemicals in processing, over hundreds of years without apparent untoward effects gives some basis for evaluating safety of these procedures. When new chemicals are proposed for introduction into foods, authorities may require extensive animal tests to provide assurance of safety—possible carcinogenicity is one aspect of the problem. Similarly, when a new processing technique which may produce chemical changes is proposed, attention must be given to possible production of carcinogens as part of the general studies on wholesomeness, or possible toxicity.

Fortunately, considerable amounts of incidental data are being accumulated on radiated foods which will provide valuable information on carcinogenicity. A number of laboratories, mainly in the U.S. Department of the Army Contract Program, are feeding radiated foods to various species in long-term experiments; records are maintained as to tumors observed. Some of this information is beginning to appear in the literature, such as the report of Poling *et al.* (5) who found the incidence of tumors to be the same on control and radiated beef diets. Our laboratory contracted to attack the problem of possible carcinogenicity specifically. There are no absolute test specifications that can be followed in this work and each case must be considered individually. However, over the years since chemical carcinogenesis was first observed in human beings and confirmed in experimental animals, various workers have evolved techniques which have come to be generally accepted as more or less standard procedure. Suggestions for general guidance have been published (6). In essence, materials are administered over long periods of time to various species and by various routes, e.g. in the diet, by skin painting, by injection or implantation, etc. The requirement for large numbers of animals per group and the long-term nature of the tests have limited most workers to the use of rats and mice. If practicable, useful additional information can be obtained by testing in other species, such as the dog. As in all tests designed to demonstrate the absence of any untoward effects of a dietary additive or a food process, it is impossible to cover every conceivable situation experimentally. One can only design experiments with the recom-

TABLE 3. TUMOR INCIDENCE IN BRAIN AND EGG YOLK PREPARATIONS (48 MICE PER GROUP—16 MONTHS)

	No. Tumors Observed					
	Fed		Injected		Painted	
	Cont.	Rad.	Cont.	Rad.	Cont.	Rad.
Mammary	7	3	0	1	4	2
Lung	3	3	0	1	15	8
Skin	0	0	0	0	3	3
Liver	0	0	0	0	0	0
Leukemia	2	0	1	0	1	4
Misc.	1	1	2	2	0	1

TABLE 4. TUMOR INCIDENCE ON BEEF AND YEAST STEROL CONCENTRATE (48 MICE PER GROUP—15 MONTHS)

	No. Tumors Observed					
	Fed		Injected		Painted	
	Cont.	Rad.	Cont.	Rad.	Cont.	Rad.
Mammary	2	3	4	2	1	1
Lung	1	2	13	12	4	7
Skin	0	0	2	4	0	2
Liver	0	0	0	3	0	0
Leukemia	1	0	5	8	3	1
Misc.	1	0	2	1	1	0

mendations of authorities in the field in mind and within the practical limitations of the means at hand so as to provide the best possible data for facilitating an end decision as to safety by the responsible authorities.

The general principles embodied in our procedures include: *a*) testing of food materials radiated at sub-sterilizing, sterilizing and above-sterilizing doses; *b*) use of both beta and gamma radiation; *c*) performance of feeding, skin painting and subcutaneous injection experiments in rats and mice; *d*) emphasis on, but not restriction to, sterol fractions; *e*) use of various strains of mice; and *f*) use of cocarcinogens in some of the tests.

Test materials have included brain and egg preparations, a milk-beef-pork-fish-cheese mixture, beef and yeast sterol concentrate mixture, peanut-corn-cottonseed oil emulsion and liquid lard. Radiation has been both beta and gamma and dosage has varied from 0.5 to 10 $\times$ 10^6 rep. Test materials are taken up in thiophene-free benzene for painting and in tricapryllin for injection, except for the liquid lard which is being injected as such subcutaneously in rats. Crude alcohol-ether extracts of brain-egg mixture and milk-meat-cheese mixture are taken up in the solvents for painting and injection. For feeding tests the materials are mixed directly with a basal diet. There are 40 rats and 48 mice in each group. Sprague-Dawley rats and Sutter, Stokely-Peterson and Jackson Laboratory (CAF_1) mice have been used to date. Croton oil is being employed as a cocarcinogen in part of the experiment with peanut-corn-cottonseed oil. Tumors observed are subjected to histopathological examination.

This is an interim report—most of the experiments are in progress. There is no apparent difference to date in incidence of tumors in the control and experimental groups. Two experiments were terminated at 15 and 16 months because of a relatively small number of survivors at that point. Numbers of tumors observed are given in tables 3 and 4. There is no marked difference in tumor incidence between control and radiated groups. Because of the occurrence of three hepatomas on the radiated injected beef and yeast sterol concentrate, additional experiments with this material have been started. It is impossible to draw any definite conclusions from the original experiment because of the small number of tumors involved.

Considering the over-all results of our experiments to date there appears to be no clear evidence of the production of carcinogens on radiation of food materials under study.

REFERENCES

(1) Read, M. S., H. F. Kraybill and N. F. Witt. Medical Nutrition Laboratory, Fitzsimons Army Hospital, Denver, Colorado, Report No. 151, 1955, "*Nutritional and Toxicological Studies on Irradiated Foods, III.*"

(2) Richardson, L. R. Progress Report V, 1956. *A Long Range Investigation of the Nutritional Properties of Radiated Food*, under contract No. DA-49-007-MD-582, Office of the Surgeon General, U.S. Department of the Army.

(3) Fieser, L. F., T. W. Greene, F. Bischoff, G. Lopez and J. J. Rupp. *J. Am. Chem. Soc.*, 77: 3928, 1955.

(4) Hieger, I. *Brit. J. Cancer* 3: 123, 1949.

(5) Poling, C. E., W. D. Warner, F. R. Humberg, E. F. Reber, W. M. Urbain and E. E. Rice. *Food Res.*, 20: 193, 1955.

(6) Burke, A. R. *Food Drug Cosmetic Law Jrl.* 10: 719, 1955.

EFFECTS OF IRRADIATION ON WHOLESOMENESS OF MIXED ORGAN MEATS IN DIET OF THE RAT[1,2]

EDWARD C. BUBL AND JOSEPH S. BUTTS, with the technical assistance of DONA K. WILLIAMS AND LOUISE S. COOPEY

From the Department of Agricultural Chemistry, Oregon Agricultural Experiment Station, Oregon State College, Corvallis, Oregon

THE USE of ionizing radiation for the sterilization of food products has a promising future. The increasingly large volume of work appearing in the literature indicates that complete sterilization can be achieved. However, accompanying this destruction of bacterial population is a companion destruction of tissue substance. This leads to flavor changes and perhaps nutritional changes due to structural variations induced by the radiation.

Before the method can be acceptable, data on the wholesomeness of irradiated foods as affecting growth, reproduction and longevity of an animal's life must be available. At the time these reported studies were begun (August 1954) a few reports concerning animal feedings were available. Unpublished data and limited circulation reports covering the work at Swift and Co., Massachusetts Institute of Technology, University of Michigan, Columbia University and the Army Medical Nutrition Laboratory were made available to the authors. In most of these reports, beef had been the food irradiated and fed. Also, in most instances the food had been irradiated using cathode rays or low intensity gamma radiation. It was therefore decided to study an organ meats mixture irradiated with high intensity gamma radiation.

In modern packing practice, the organs, such as the kidney, heart, brain and tripe, are utilized as the fresh product or, in the case of heart and tripe, in the preparation of table-ready meats. These organs, as they contain appreciable levels of sterols, vitamins, nucleic acids and other metabolic products, might be subject to profound changes during irradiation. The effects of feeding an irradiated organ meats mixture through a four-generation life span in rats is reported here.

EXPERIMENTAL

The organ meats used in the ration were obtained from a local slaughterhouse. They were all from the beef animal. The composition of the mixture is shown in table 1. This composition was selected to give a broad selection of organs containing a high protein content. The organs were washed, weighed, then ground in a sausage grinder using the fine plate. The ground mass was then thoroughly mixed in a sausage mixer. The mixture was then packed into #2 (307 x 405) 'C' enamel cans, sealed and sharp frozen. The cans were divided at random—one half for the control ration and one half for the irradiated ration. The cans for the irradiated ration were irradiated to 3×10^6 rep in the canal of the Materials Testing Reactor, Phillips Petroleum Co., Idaho Falls, Idaho. These cans were shipped and irradiated in the frozen state. All the cans were stored in the deep freeze (−20°C) until mixed into the fed ration. The irradiated product was normal in color, but had an intense and characteristic irradiated odor.

The ration as fed is tabulated in table 2. The other water-soluble vitamins and vitamin K were mixed directly into the ration at levels previously reported (1). Vitamns A, D and E were given orally twice weekly at a minimum level of 50 U.S.P. units A, 5 U.S.P. units D and 9 mg *dl* α-tocopherol (9.9 IU) per administration.

It was calculated, using the work of Schweigert (2), that the level of methionine in the diet was low. Therefore, crystalline methionine was added. The addition of guar gum gave a ration with superior handling properties and greatly reduced spillage losses (personal communication from E. E. Rice).

Fifty-six 28-day-old rats of the Wistar Strain

[1] This paper reports research undertaken in in cooperation with the Office of the Surgeon General under Contract DA-49-007-MD580.

[2] Technical Paper No. 975, Oregon Agricultural Experimental Station.

TABLE 1. COMPOSITION OF ORGAN MEATS MIXTURE

	%		%
Beef kidney	21.0	Beef brain	6.5
Beef heart	25.0	Beef tripe	15.0
Beef spleen	25.0	Beef pancreas	5.0
Beef spinal cord	2.5		

TABLE 2. DIET AS FED ANIMALS

	%		%
Mixed organ meats (wet)	60.0	Choline chloride	0.3
		Inositol	0.2
Casein	10.0	Liver extract (Wilson's 1:20)	0.5
Potato starch	23.4		
Guar gum	1.0		
Salts (Jones + Foster)	4.0	Methionine	0.6

Other water soluble vitamins and K are mixed in ration. A, D, E, given orally.

TABLE 3. AVERAGE WEIGHTS IN GRAMS, ORGAN MEATS DIET

	1st Generation		2nd Generation		3rd Generation	
	Irr.	Control	Irr.	Control	Irr.	Control
A. Males						
No. of Animals	13	13	9	9	15	17
Days						
0	41	40	47	50	38	43
20	112	112	136	143	136	132
40	183	183	226	236	229	224
60	222	233	296	293	289	288
80	268	274	324	324	327	324
100	292	303	342	344	349	351
B. Females						
No. of Animals	14	14	9	9	15	13
Days						
0	38	38	47	49	38	42
20	100	100	113	118	112	108
40	150	152	167	159	162	154
60	178	171	192	191	187	181
80	195	194	204	205	206	195
100	209	208	216	220	218	207

comprised the first generation. Four litter mate animals, 2 males and 2 females, were selected from each of 14 litters, with as uniform weights as possible. One male and one female were placed on the irradiated ration; their companion litter mates were fed the control ration.

The animals were housed individually and were allowed food and water ad libitum. Animal weights were recorded at 5-day intervals, food intake was recorded daily. These data were collected during a 100-day growth period.

After the completion of this period the female animals were bred. Each female was mated with at least 3 different males from the same food group. Each male was housed with the female for a 6-day period before being rotated to another female. The fertility and litter-rearing capacity were recorded. The females of the first generation were rested 3 weeks, then bred again using the identical pattern.

A selection of 36 litter mate animals, 1 male and 1 female from each of the females producing second litters, made up the second generation. These were grown and bred as above. The second generation was, however, bred only once.

Sixty pups from the second generation females, selected as above, were raised and bred as the third generation. The adult females were bred to produce the fourth generation which was weaned at 28 days when the experiment was terminated.

Some of the adult animals of the first generation were kept as a longevity group. Sixteen animals, 4 males and 4 females, from each of the two food groups comprised this group. The remainder of the adult animals were killed for gross pathological examination.

TABLE 4. SUMMARY OF BREEDING RECORDS

	1st Generation		2nd Generation		3rd Generation	
	Irr.	Control	Irr.	Control	Irr.	Control
No. females bred	14	14	9	9	14	13
Total no. pups born	145	155	87	85	138	112
Total no. pups weaned	102	126	74	67	124	80
Average no. born	10.4	11.9	9.4	9.5	10.6	10.1
Average no. weaned	9.3	10.5	8.2	7.0	9.5	7.2
Average wt., gm	30.5	30.8	29.2	24.5	36.0	25.3
No. sterile females	0	1	0	0	1	2

TABLE 5. SUMMARY OF MORTALITY AND SACRIFICE OF FIRST GENERATION ANIMALS

	Irradiated		Control	
	Males	Females	Males	Females
No. of animals started	14	14	14	14
Losses 0–6 months	1	0	2	0
Losses 6–12 months	1	0	0	0
Sacrificed animals	8	9	7	10
Losses 12–18 months	1	1	1	0
Losses 18–21 months	0	2	1	1
Survivors	3	2	3	3

RESULTS AND DISCUSSION

The data obtained on the first, second and third generation animals during their growth periods are shown in table 3A and B. It can be seen from these data that the irradiation of the organ meats has no effect upon the growth of the animals. The food intake data for both groups show equal intake and utilization. One difference noticed was that the second and third generation male animals consumed more ration, but it was also evident that they made much superior growth.

The breeding performances of the females from either of the food groups also showed comparable patterns. These data are shown in table 4. It was our policy to allow the female to keep all the pups she bore rather than cut the litters down to a uniform number. We felt that this method placed more stress upon the mother and would give us a better picture of the adequacy of the diet. In general, the litter size and number of animals weaned was much better on the organ meats rations than on our stock colony ration.

In table 5 a summary of the first generation longevity group is given. There were 11 of the 16 animals still alive at 21 months from birth. These animals will be allowed to continue to 24 months. All the surviving animals or those sacrificed in the intervening time will be examined histopathologically.

All of the pups and adults that were discarded were examined for gross pathology. Abnormal tissues were prepared for future histopathological examination. However, a very low percentage of the animals had abnormalities of any kind and only one tumor has been found.

SUMMARY

A two-year, four-generation, feeding, breeding and longevity study on rats fed irradiated or non-irradiated organ meats diet is reported. Four generations of animals were fed these diets without adverse effects upon growth or reproduction. Although the longevity period has not been completed, little difference can be seen between the irradiated and the control animals.

REFERENCES

1. Bubl, E. C. and J. S. Butts. *J. Biol. Chem.* 180: 839, 1949.
2. Schweigert, B. S., B. A. Bennett and B. T. Guthneck. *Food Res.* 19: 219, 1954.

WHOLESOMENESS OF GAMMA-IRRADIATED FOODS FED TO RATS[1]

H. F. Kraybill, Merrill S. Read and T. E. Friedemann

From the U. S. Army, Medical Nutrition Laboratory, Denver, Colorado

Radiation sterilization has been widely recognized as a promising new method for the preservation of food items. It is anticipated that, once the complex technological problems relating to off odors, flavors and abnormal textures have been solved, this revolutionary process will permit the storage of many foods for an extended period of time with a minimum of refrigeration. However, before radiation sterilized foods may be consumed safely, we must be certain through exhaustive preliminary animal experiments that the irradiated products do not exert deleterious effects upon growth, reproduction or total life span. These biological criteria designed to establish the safety characteristics of a product are useful in determining the wholesomeness of irradiated foods.

Wholesomeness encompasses at least three areas of interest: *1*) nutritional adequacy, *2*) toxicity and *3*) carcinogenicity. Although these are difficult to separate experimentally, insofar as possible they must be investigated individually in order to obtain definitive information about the safety and suitability of feeding irradiated foods to human populations. The variety of papers presented at this symposium further emphasizes the many possible approaches for the comprehensive investigation of each phase of wholesomeness. It is only through the application of many research techniques that a complete picture of the effects of radiation sterilization on food items will be obtained.

Due to the newness of irradiation sterilization as a method of food preservation, specific mechanisms for radiation induced reactions are still notably lacking. An excellent review of this area has been prepared by Proctor and Goldblith (1), however. Typical reactions during the irradiation of pure solutions include the deamination (2, 3) and decarboxylation (3) of amino acids, the oxidation of fats to peroxides (4, 5), and the destruction of vitamins (4, 6, 7). Proteins are more resistant to ionizing radiation than amino acids (8) although even they can be altered by sufficiently large irradiation dosages (4). In all cases, the nature and the extent of degradation is dependent upon the conditions (i.e. pH, presence of oxygen, temperature, etc.) as well as on the presence of other compounds in the system which preferentially absorb the incident energy so as to afford protection for the nutrients. It is thus readily apparent that each food item must be considered a separate chemical system from the viewpoint of the wholesomeness of the irradiated product. Furthermore, due to the small magnitude of the chemical changes accompanying radiation sterilization, the animal-feeding experiments ordinarily employed must be expanded to include more sensitive criteria, such as have been outlined in two recent articles (9, 10).

One of the earliest wholesomeness studies on an irradiated diet was reported by DaCosta and Levenson (11) in 1951. Employing a semi-synthetic diet, these authors found no deleterious effects on growth but did observe a decrease in fertility among rats fed the irradiated ration. Satisfactory growth has also been obtained in short-term (5–8 weeks) rat-feeding experiments employing irradiated cooked meat (12), milk and milk products (4, 13), or egg powder (14). A comprehensive investigation employing successive generations of rats raised on irradiated beef has been reported by Poling *et al.* (15). The latter workers observed a decrease in fertility among animals fed the irradiated ration analogous to that previously reported by DaCosta and Levenson (11). It was corrected by supplementation with vitamin E. No other serious loss of nutritional quality nor occurrence of any toxic properties was reported. In a comparative study using a semi-synthetic ration sterilized with steam or cathode-irradiation, Luckey *et al.* (16)

[1] This paper represents work done on a collaborative project between University of Colorado and Medical Nutrition Laboratory in cooperation with the Office of The Surgeon General under Contract DA-49-007-MD-549.

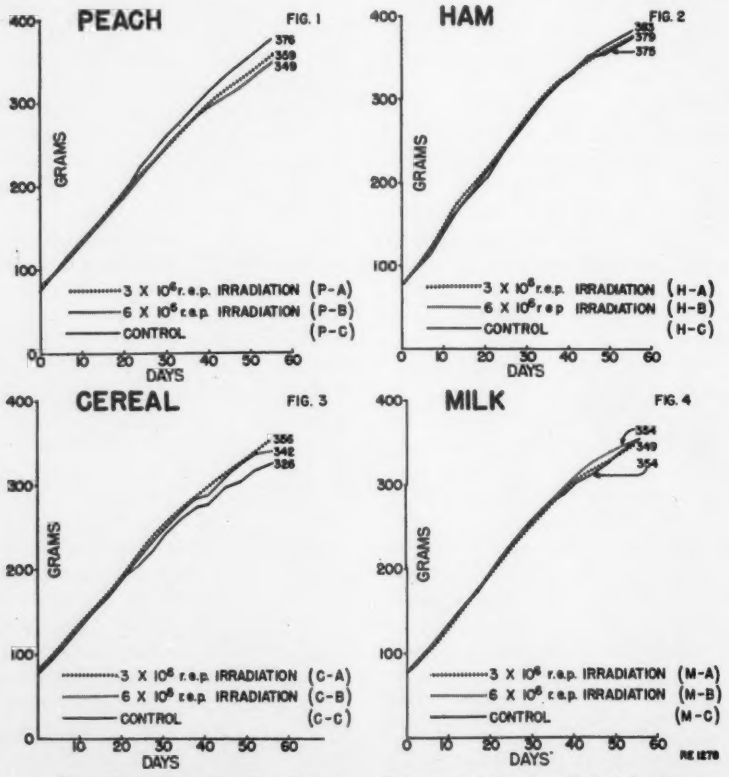
found that mice fed the irradiated ration grew as well as those on the other diets. In general, vitamin losses were less in the gamma-irradiated than in the steam-sterilized product.

The Medical Nutrition Laboratory is primarily interested in the physiological aspects relative to the feeding of radiation-sterilized foods. As an unavoidable corollary, we are also investigating to a lesser extent the question of nutritional adequacy of several irradiated food items. Our program may be divided into two primary phases: *1*) feeding individual food items to rats for relatively short periods (8–12 weeks), and *2*) the long-term feeding of a diet composed entirely of irradiated food items to four successive generations of rats. The first phase, the short-term studies, has been essentially completed, whereas the second, or long-term studies, is still in progress.

In the short-term studies, the question of toxicity was separated from nutritional adequacy by compounding a diet containing 65% dry weight of a nutritionally adequate basal ration and 35% dry solids of the food to be tested. This diet contained a minimum of 15% protein and 10% fat. Non-irradiated vitamin supplements were also incorporated in the diet. For each study, individual food items were gamma-irradiated to a total dosage of three and six megarep. These irradiation levels were selected as representative of the normal dosage anticipated for commercial processing or sterilization and twice this dosage to augment the production of potentially toxic radiation by-products. Comparable non-irradiated foods were used in the control diets.

Our experiments were conducted starting with weanling male albino rats. Daily food consumption records were maintained throughout the eight-week feeding study and biweekly weighings were taken as an index of growth. At the end of the experiments, representative animals were autopsied for histopathological examination.

Twelve foods have been investigated by this



FIGS. 1–4. Growth rate of rats on diets containing irradiated foods

technique: ground beef, fresh ham, sliced bacon, haddock fillet, green beans, whole kernel corn, sliced beets, frozen strawberries, sliced peaches, bread, military cereal bar and powdered whole milk. Excellent growth and food consumption was obtained on all diets at the two irradiation levels. Representative growth curves for four foods are shown in figures 1–4. Gross observations as well as the histopathological examination of representative tissues indicated that all animals raised on all the diets were in good health and had demonstrated satisfactory growth and development. Statistical analysis of total growth of animals fed the non-irradiated and irradiated diets also have been completed. The results of these investigations indicated that irradiation sterilized foods were equally as satisfactory as non-irradiated foods insofar as growth and feed efficiency were concerned.

The second phase of our toxicity investigations consisted of a long-term successive generation feeding study employing both male and female rats. The diets used in these studies were compounded from nine foods previously found to be non-toxic in the short-term studies. The foods used were: ground beef, haddock fillet, fresh ham, sliced bacon, peaches, powdered whole milk, beets, green beans and military cereal bar. These were individually gamma-irradiated in cans to a dosage of six million rep. Comparable non-irradiated foods were used in the control diets. The foods were compounded in the diets so as to provide adequate levels of protein, fat and carbohydrate necessary for optimum growth and lactation. Non-irradiated vitamins were added to each diet to preclude any possible micronutrient deficiency.

Records of food consumption and weight gain were maintained during growth in the same manner as in the short-term studies. Growth curves obtained on the parent generation show no differences in the growth of male or female rats fed the irradiated or non-irradiated diets for 80 days (fig. 5). Similar results have been obtained with the animals of the second generation.

Through an appropriately planned breeding program it has been possible to obtain fertility figures for both females and males on each diet as well as reproduction and lactation data for the females. It is difficult to evaluate data on fertility of female rats since much is dependent upon male fertility information. However, it has been observed in the experiments thus far that consumption of the irradiated diet does not greatly change the fertility of female rats compared to female rats raised on the non-irradiated diet. To indicate how closely the two groups of females correspond to each other, we have summarized some information on the first litters

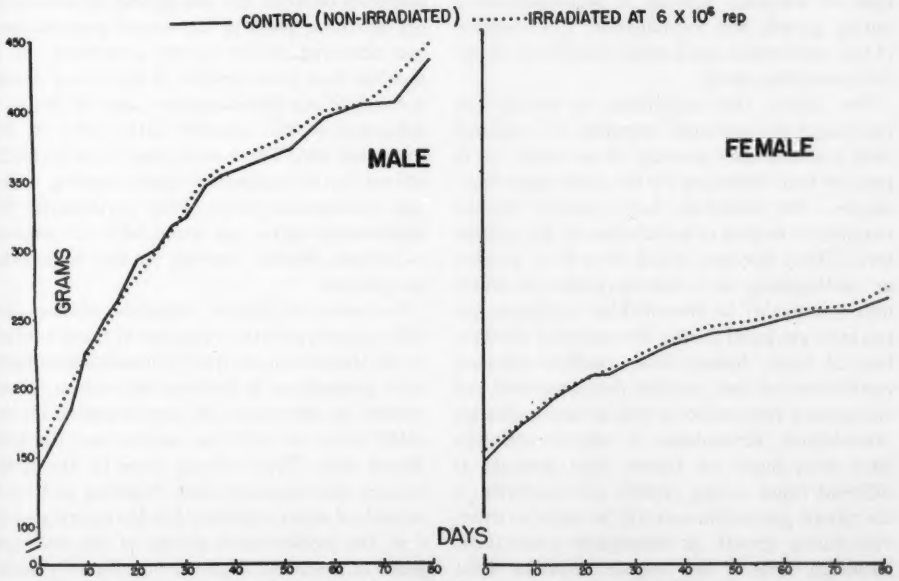


FIG. 5. Growth rate of albino rats on composite diets

TABLE 1. FEMALE REPRODUCTION AND LACTATION DATA FROM FIRST LITTERS OF THE PARENT GENERATION RAISED ON COMPOSITE NON-IRRADIATED AND IRRADIATED DIETS

	Non-Irradiated		Irradiated	
No.	No.	%	No.	%
Pups Counted	144		146	
Alive	127	88	122	84
Dead	17	12	24	16
Total pups born per litter	9.0		9.7	
Per litter weaned	6.8		6.4	
Av. Weight Per:				
Pup born	6.0 gm		6.1 gm	
Pup weaned	29.5 gm		29.2 gm	

from the parent generation (table 1). It is of interest to note that the total number of pups born was about the same for each group of females and that the birth weights of these pups were also nearly equal. However, the percentage of live births was slightly higher in the control group than in those fed the irradiated diet. Lactational performance also was nearly equal in the two groups, both with regard to numbers of animals weaned per litter and to the average weight of the pups weaned.

The information on the fertility of males suggests that a higher incidence of sterility occurred in males fed the irradiated diet than in those fed the non-irradiated diet. This is in spite of adequate vitamin E supplementation during growth and reproduction. Confirmation of this observation must await completion of our four-generation study.

We believe that alterations in metabolism resulting from continued ingestion of irradiated food products may possibly be so subtle as to prevent their detection by the usual gross techniques. We, therefore, have selected several enzymes as indices of metabolism at the cellular level. These enzymes, which have been selected as participating in metabolic pathways which may conceivably be disrupted by irradiation by-products produced during the radiation sterilization of foods, include liver xanthine oxidase, cytochrome oxidase, succinic dehydrogenase and endogenous respiration as well as serum alkaline phosphatase. Estimations of enzyme activities have been made on tissues from animals at different times during growth and longevity in the parent generation and will be made at intervals during growth in subsequent generations. Although some of the enzyme activities differ between animals raised on the irradiated and

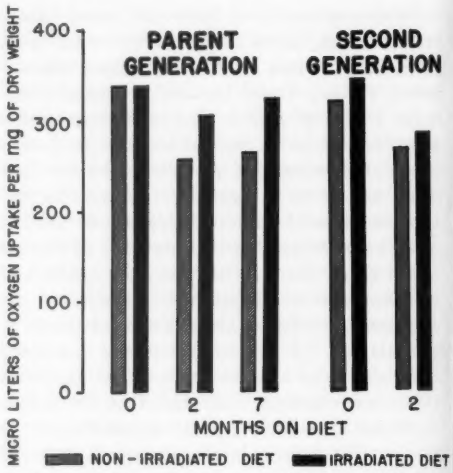


FIG. 6. Liver cytochrome oxidase activities in male rats maintained on non-irradiated and irradiated diets.

non-irradiated diets, it is still too early in the experiment to draw broad conclusions. Nevertheless, we do feel that the data obtained from liver cytochrome oxidase determinations are of interest (fig. 6). There appears to be a systematic increase in the activity of this enzyme in those animals fed the irradiated diet. It should be pointed out, however, that the difference in activities between the two groups of animals is not nearly so great in the second generation as was observed in the parent generation. It is possible that these results, if confirmed during the rest of our investigations, may be due to a deficiency of the essential fatty acids in the irradiated diet. These acids may be structurally altered by the oxidations accompanying radiation sterilization, since fats, particularly the unsaturated types, are quite labile to ionizing radiations. Further studies on this hypothesis are planned.

In order to further establish whether the differences in growth or changes in tissue enzyme levels that occur are due to irradiation-induced toxic products or to nutrient destruction, it was decided to determine the metabolizable energy (ME) value of both the control and the irradiated diets. This is being done by the bomb balance technique for both weanling and adult animals of each generation. For the parent generation, the metabolizable energy of the diet composed of irradiated foods was significantly higher than that of the control diet. Although the

studies have not been completed on the animals of the second generation, calculations to date indicate a similar relationship. The increased ME appeared to be due to a greater apparent digestibility of protein and perhaps of carbohydrate in the radiation sterilized diet.

From our results to date it would appear that radiation sterilized foods are not toxic when individually fed to rats for periods of eight weeks. Furthermore, continued consumption of a 100% irradiated diet through two successive generations of rats has also shown that irradiated foods are non-toxic. The slight alterations in metabolism observed in the rats fed irradiated food for long periods may be related to marginal quantities of essential nutrient requirements rather than any assumed effect from potential toxic products. In any case the critical evaluation of our data must await the completion of the four-generation feeding study.

REFERENCES

1. Proctor, B. E., and S. A. Goldblith. Tech. Rpt. #1: NYO 3337, 1952.
2. Barron, E. S. G., and P. Johnson. *Rad. Res.* 2: 145, 1955.
3. Sharpless, N. E., A. E. Blair, and C. R. Maxwell. *Rad. Res.* 2: 135, 1955.
4. Kung, H. C., E. L. Gaden, Jr., and C. G. King. *J. Agr. Food Chem.* 1: 142, 1953.
5. Mead, J. F. *Science* 115: 470, 1952.
6. Proctor, B. E., and S. A. Goldblith. *Nucl.* 5: 56, 1949.
7. Markakis, P. C., S. A. Goldblith, and B. E. Proctor. *Nucl.* 9: 71, 1951.
8. Bhatia, D. S., and B. E. Proctor. *Biochem. J.* 49: 550, 1951.
9. Lehman, A. J., W. I. Patterson, B. Davidson, E. C. Hagen, G. Woodard, E. P. Laug, J. P. Frawley, O. G. Fitzhugh, A. R. Bourke, J. H. Draize, A. A. Nelson, and B. J. Voos. *Food Drug Cosmetic Law Journal* 10: 679, 1955.
10. Lehman, A. J., and E. P. Laug. *Nucl.* 12: 52, 1954.
11. DaCosta, E., and S. M. Levenson. U. S. Army Medical Nutrition Laboratory Report #89, 1951.
12. Brownell, L. E. *Utilization of Gross Fission Products*, Prog. Rpt. #5, Sept. 1953.
13. Brownell, L. E. *Utilization of Gross Fission Products*, Prog. Rpt. #2, Jan. 1952.
14. Proctor, B. E., and H. Sherman. *Quart. Prog. Rpt.* March 1955.
15. Poling, C. E., W. D. Warner, F. R. Humburg, E. F. Reber, W. M. Urbain, and E. E. Rice. *Food Res.* 20: 193, 1955.
16. Luckey, T. D., M. Wagner, J. A. Reyniers, and F. L. Foster. *Food Res.* 20: 180, 1955.

THE AMERICAN PHYSIOLOGICAL SOCIETY

Symposium on Biology of Aging[1]

Chairman: N. W. SHOCK

AGING AS A BIOLOGICAL PROBLEM

N. W. SHOCK

From the Gerontology Branch, National Heart Institute, National Institutes of Health, Bethesda, and the Baltimore City Hospitals, Baltimore, Maryland

AGING IS A PHENOMENON which is apparent to all of us either as an individual personal experience or as changes in the society in which we live. The phenomena of aging give rise to many problems (1). Some of them are forced on the attention of physicians by virtue of the ages of the patients whom they see in their offices and in their hospital beds (2). Personnel officers are confronted with other aspects of the problem in hiring new workers and retiring older ones (3). Social workers are aware of the pressures generated in our society by the increased number of elderly people and see the need to supplement income (4), find appropriate housing (5), and provide for their activities (6–8). Physiologists are being pressed to devise indices of 'physiological age' which can serve as a yardstick to determine the time of retirement of individual workers (9). These are all important problems and a great deal has been written about the urgency and need for action. Important as these problems may be, they will not be considered in this Symposium which has been organized with the conviction that the study of aging must not only serve as a foundation for the ultimate solution of the practical problems of gerontology and geriatrics, but is also a biological phenomenon worthy of scientific study in its own right. It is our aim to consider briefly not only some of the known facts about age changes in the structure and function of cells and tissues but, more important, to discuss new concepts, approaches and techniques which may be brought to bear on the problems of aging.

For the purposes of our discussion, a number of arbitrary definitions are proposed. First of all, we will regard aging as a process which goes on in all living things with the passage of time; i.e., by aging we mean simply the changes that occur in living things with the passage of time (10, 11). Obviously, this will include the period of growth and development as well as maturity and the later parts of the life span.

Biologists have devoted their primary efforts to the study of differentiation and growth—the early phases of aging. Minot (12) made the valid observation that the rate of aging, judged by the decline in growth rate, was greatest in the embryo and least in old age. Although this generalization has become a major premise of gerontology, it has not proved very useful in understanding what happens after a stable size and form is attained in the adult animal (13). In spite of the connotations implied by the word, it may be better to reserve the term 'senescence' for this later period of the life span.

If we start with a large population of animals and simply tabulate the number remaining alive after a given interval of time, we obtain the well-known survival curve (13). Under certain conditions, and for some species of animals, the num-

[1] Atlantic City, April 17, 1956.

ber of animals dying within a specified time interval is a maximum at early ages and gradually decreases throughout the remainder of the life span. This type of curve is found in many species of animals in the wild state (14, 15), and is presumed to describe the death rate in early man (16). Under these circumstances, the phenomenon of senescence does not appear because of the large losses which are distributed by chance among the young. However, in laboratory animals and in man, the survival curve approaches a rectangular form; i.e., the number of animals dying at early ages is small and increases with age. If now we plot the proportion of animals dying within a specified time interval against age, we obtain the well-known mortality curve (17, 18). Medawar (19) and Comfort (14) have called the ratio of the number of organisms that die within any chosen interval of age over the number of organisms alive at the beginning of the interval, a measure of the 'force of mortality'. It is this form of mortality curve which leads to the generalization that the probability of death increases with age. The 'force of mortality' is a statistical construct which may be useful in assessing the effectiveness of genetic composition, temperature, nutrition, etc., in altering longevity in a population. Thus, senescence refers to the decreasing ability to survive stress, injury or changes with increasing age.

Among inanimate objects such as test tubes, the probability of breakage during any specified interval of time is constant, assuming uniform usage. The 'mortality curve' is a straight line with a slope equivalent to the mean rate of breakage per unit of time. In contrast, in most adult animals, including man, the probability of dying within the next year increases steadily with age. The goal of research on senescence is to understand the reasons why this is so.

Although the mortality curve can give important information about the 'force of mortality' in a total population, it cannot specify the 'vitality' or health status of the individual living members of the population (20). We must turn to other indices of aging or senescence if we are to evaluate the status of individuals.

The fact that the probability of death increases with age leads us to the presumption that some changes occur in the individual animal from year to year or from moment to moment. Our problem is, first of all, to identify and describe the changes that occur in the organism with the passage of time. It is obvious that two factors may contribute to the increased susceptibility to dying: first, there may be innate or built-in age changes (11, 21, 22) or, secondly, some of the effects of reduced vitality may be the result of the current or accumulated effects of stress, injury etc., to which the animal has been exposed throughout its life career (23, 24). Thus, the study of senescence must take into account what has happened to the animal in the preceding phases of its life (25). Aging is a process. Its study and characterization require observations on the animal throughout its life span. We cannot expect to understand aging if we study only senescent animals. Ideally, we should make repeated observations on the same animal throughout its entire life span (1). This is a difficult assignment which can be met only in studies of species where the total life span is less than that of the investigator. It is also impossible, in such longitudinal studies, to make observations that require sacrifice of the animal. Thus, many studies must first be carried out by the cross-sectional method, where different animals constitute each age group. Often we can get good first approximations by comparing groups of animals at two or more points in their life span. To gain information about senescence, we must be certain that measurements are made at least at two points beyond the period of rapid growth in the animal.

The attempt to distinguish between 'pure aging' and 'pathology' has provoked discussions that generate more heat than light. There is also the problem of semantics, since what the anatomist or histologist may describe as an age change may later become identified with a describable disease state or syndrome and thus be relegated to pathology (26). If we consider disease states as instances of altered or deranged metabolic processes in cells and tissues, our methods of approach will not be greatly altered whether we insist on the study of 'pure aging' or are willing to accept 'pathology' as part of aging or senescence.

With increasing age, there are demonstrable anatomical changes in the tissues and organs of most animals. Histologically, a reduction in the number of cells, particularly in the nervous system, but also in other tissues that have lost their ability to divide and multiply may be seen with increasing age. Aged tissues show a decrease in the size of the functioning parenchymal cells with an increase both in amount and density of the interstitial substances (27, 28). An astute housewife can distinguish between cuts of beef from an

old steer and a young calf without benefit of microtome or microscope. These anatomical facts have an important bearing on the observations of physiologists which demonstrate a reduction in physiological capacities and functions with increasing age (10). To what extent can the physiological impairments of old age be explained simply by a reduction in the amount of functioning protoplasm, or number of functional cells remaining in the organism? All too often alterations in the metabolism of the total organism have been interpreted as indicating a reduction in cellular function. Thus, one of our problems in the study of aging animals is to find an index of the amount of functioning protoplasm that is present. In the past, surface area has been used as a common denominator, but is this an adequate index for the study of aging? Results from our own studies on basal metabolism in men raise doubts (25). This is a problem that must also be faced by the biochemist in characterizing the metabolism of tissues and cells.

Even if the loss of functioning cells is a primary factor in the senescence of metazoans, we still need to determine the conditions responsible for the failure of these cells to maintain themselves. Impairment of blood supply may play a role in some animals, but this can hardly be the total answer, since senescence and death occur in animal species that are devoid of a vascular system. Fundamentally, the question is whether cellular atrophy rises from alterations in metabolic processes within the cell or whether it is the result of changes in the cellular environment. Another possibility that should be explored is that alterations in the characteristics of cell membranes interfere with the adequate exchange of substances. Perhaps more attention should be paid to the characteristics of the extra-cellular matrix—the ground substance. If aging is associated with the impairment of some specific enzyme system of the cell, an accumulation of the intermediary metabolite, preceding this enzyme reaction, should be demonstrable. With our knowledge of metabolic pathways in cellular metabolism, we ought to find out whether or not there is any shift in the major pathways in old cells as compared with young. With current advances in technology, there is no reason why these questions should not be investigated.

It is often charged that research on aging suffers from the lack of any theoretical formulation. On the contrary, it seems that gerontology suffers from a plethora of hypotheses. Unlike other fields of biology, where it is possible to present the major theories historically and to show a steady progression from a large number of speculative to one or two main hypotheses, theories on senescence can only be grouped or catalogued. Although the terminology has changed, the basic hypotheses of aging have scarcely altered from those offered by Aristotle. Most theories of senescence attribute the phenomenon either to the gradual accumulation of some toxic material or the exhaustion of an essential substance. Superimposed on either of these assumptions is the presumption that aging is either the expression of some 'built-in' process or the gradual accumulation of insult and injury. As has been indicated before, senescence is no doubt the result of both processes, since the very act of living must expose the animal to some insult and injury. Our greatest need is to develop testable hypotheses.

In my opinion, the identification of 'aging' or 'senescence', with a decline in physiological or biological functions, begs the question. Although data from mortality curves indicate an increasing probability of death within a population, they do not offer any information about the characteristics of the individuals who do not die. This is the question we wish to answer. I believe it is ill-advised to make a prejudgment of decline—it is better to look for differences, without specifying their direction.

In contrast to growth and development, senescence has not been a phase of aging which has attracted many biologists as an area for systematic research. Consequently, a large proportion of the information which we do have has been acquired largely as a by-product of other studies. In view of the difficulties in obtaining aged organisms for study, plus the common belief in the inevitability of senescence and even its biological necessity, this is easy to understand. However, with the technological developments in science and changing attitudes about aging, it is my conviction that more investigators will develop an interest in research on aging, and that basic studies will be richly rewarding.

From what has been said, it is clear that a wide variety of techniques needs to be applied to the problem. Although Pearl (21) and others have demonstrated the importance of genetics in the determination of longevity, can we say that we have exhausted the possibilities of studies in this area of biology? It may well be that important

clues for further research might be uncovered if we knew more about the 'force of mortality' in animals other than flies, rats, mice and men.

It is also clear that detailed anatomical physiological and biochemical studies are needed on tissues and cells of many different species of animals in view of the wide differences in longevity which exist. We already have a good many isolated facts about senescent animals available to us, but all too often the observations cannot be compared because of differences in methodology and technique. It is the goal of this Symposium not only to review briefly the status of our knowledge in some of these areas but, more importantly, to discuss concepts and to gain from the knowledge and experience of all of you an insight into new approaches, new strategy and new tactics.

REFERENCES

1. Shock, N. W. *Trends in Gerontology*. Stanford, Calif.: Stanford Univ. Press, 1951.
2. Monroe, R. T. *Diseases in Old Age*. Cambridge: Harvard, 1951.
3. Breckinridge, E. L. *Effective Use of Older Workers*. Chicago: Wilcox and Follett Company, 1953.
4. Corson J. J. and J. W. McConnell. *Economic Needs of Older People*. New York: Twentieth Century Fund, 1956.
5. Donahue, W., *Editor. Housing the Aged*. Ann Arbor: Univ. Michigan Press, 1954.
6. Breckinridge, E. *Community Services for Older People: The Chicago Plan*. Chicago: Wilcox and Follett Company, 1952.
7. Kaplan, J. *A Social Program for Older People*. Minneapolis: Univ. Minnesota Press, 1953.
8. Friedmann, E. A. and R. J. Havighurst. *The Meaning of Work and Retirement*. Chicago: Univ. Chicago Press, 1954.
9. Mathiasen, G., *Editor. Criteria for Retirement*. New York: Putnam, 1952.
10. Shock, N. W. *Bull. New York Acad. Med.* 32: 268, 1956.
11. Lansing, A. I. *Bull. New York Acad. Med.* 32: 5, 1956.
12. Minot, C. S. *The Problem of Age, Growth and Death, A Study of Cytomorphosis, Based on Lectures at the Lowell Institute, March 1907*. London and New York: Putnam, 1908.
13. Lansing, A. I., *Editor. Cowdry's Problems of Ageing, Biological and Medical Aspects*. Baltimore: Williams & Wilkins, 3rd ed., 1952.
14. Comfort, A. *Biol. Rev.* 29: 284, 1954.
15. Comfort, A. *The Biology of Senescence*. New York: Rinehart, 1956.
16. Macdonnell, W. R. *Biometrika* 9: 366, 1913.
17. Simms, H. S. *J. Gerontol.* 1: 13, 1946.
18. Dublin, L. I., A. J. Lotka and M. Spiegelman. *Length of Life*. New York: Roland Press, 1949.
19. Medawar, P. B. *An Unsolved Problem of Biology*. London: Lewis, 1952.
20. Davies, D. F. *J. Gerontol.* 9: 186, 1954.
21. Pearl, R. *Quart. Rev. Biol.* 3: 391, 1928.
22. Child, C. M. *Senescence and Rejuvenescence*. Chicago: Univ. Chicago Press, 1915.
23. Warthin, A. S. *Old Age, the Major Involution; the Physiology and Pathology of the Aging Process*. New York: Hoeber, 1929.
24. Metchnikov, E. *The Prolongation of Life; Optimistic Studies*. New York and London: Putnam, 1908.
25. Jones, H. B. *Trans. N. Y. Acad. Sci.* 18: 298, 1956
26. Shock, N. W. *In: Symposium on Problems of Gerontology*. New York: Natl. Vitamin Fndn., Nutrition Symposium Series No. 9, 1954.
27. Oliver, J. *In:* E. J. Stieglitz (Ed.), *Geriatric Medicine*. Philadelphia: Lippincott, 1954.
28. Andrew, W. *Cellular Changes with Age*. Springfield, Ill.: Thomas, 1952.

AGE CHANGES IN THE MORPHOLOGY OF TISSUES AND CELLS

WARREN ANDREW

From the Department of Anatomy, Bowman Gray School of Medicine, Winston-Salem, North Carolina

THE CONCEPT OF SENILE CHANGE in the various organs which is most widely accepted by pathologists and physicians is one which may be described under the general term 'senile fibrosis'. The parenchyma, composed of the essential functioning cells of an organ, is supposed to undergo atrophy and to be replaced gradually by the growth of fibrous connective tissue. Organs thus affected show a loss of their original softness and pliability and acquire a firm, dense character.

This view goes back at least as far as Metchnikov (1). He described senile change as beginning with infiltration of an organ by phagocytic cells which consume the parenchymal cells little by little and form the new connective tissue. The idea of this type of phagocytic activity has been rejected but the second part of the concept, the substitution of connective tissue for parenchyma, remains as a prevalent view.

Is this concept of senile fibrosis an over-simplification? Is it a true description of 'normal' senile change or is it a pathological alteration? How widespread will such a process be found to be in relatively healthy senile animals sacrificed for study as compared to human autopsy material, where frequently multiple pathological factors must be considered? These are some of the questions which confront us when we attempt to present a description of 'age changes in the morphology of tissues and cells'.

As a matter of fact, the organs upon which many of the principal arguments for senile fibrosis were based originally were the large glandular ones such as the liver, kidneys and pancreas.

In the course of our own investigations on senile change we have had the opportunity to study a large number of different organs, representing various of the major systems, in pedigreed animals, particularly mice of the C57 Black stock and Wistar Institute rats. The mice have been kept in our own animal rooms, at least from late maturity, about 400 days of age, up into senility, while the organs of the rats have been shipped to us after fixation at the Wistar Institute.

In addition to the animal material, we have studied human organs from both autopsy and surgical material.

Let us discuss now some of the findings in the tissues which we have studied.

The liver (fig. 1 *A*, *B*) shows definite changes in senility (2). While these present a consistent pattern, they are not generally of a fibrotic type and are apparent only if some attention is being paid to cellular detail. The hypertrophy and hyperchromatism of individual nuclei, and the occurrence of intranuclear inclusion bodies, are the outstanding features in old animals. The intranuclear inclusions have been studied by Wilson (3) by chemical and other means. He feels that they are derived from nucleoli, thus agreeing with Findlay (4) and ourselves (2). Cytoplasmic changes occur and may well be considered degenerative in nature but there is little actual loss of cells and little replacement of parenchyma by connective tissue. An important point to stress here, we believe, is that we are dealing with animals of maximum age.

There are two other types of change which have been described for the liver in old age. The first of these is lymphocytic infiltration of the periportal space. This phenomenon we have found to be very common in the livers of old mice, rats and human beings (2, and unpublished data). It seems to constitute a sort of colonization of areas of the liver by the lymphocytic cells which at times replace considerable areas of hepatic parenchyma. The process occurs also in a variety of conditions other than old age (5).

The second phenomenon, of less frequent occurrence, is proliferation of bile ducts. Such proliferation was found by Korenchevsky, Paris and Benjamin (6) in about 18% of senile rats which were being used as controls in an endocrinological experiment. We have not as yet made observations on this type of change in the liver but have found a marked proliferation of ducts in the pancreas in many senile rats (7).

Turning now to this second large gland, we may consider how the picture of the pancreas in old age corresponds to a concept of 'senile fi-

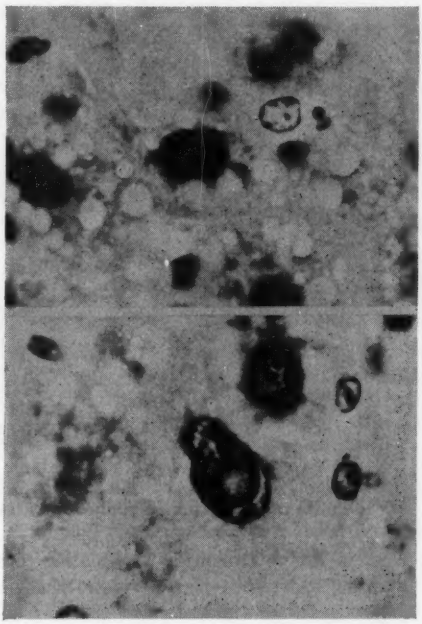


FIG. 1 (*above*). Liver of a senile mouse—811-day-old female. In old animals a much greater variety in size and shape of nuclei is seen than in young ones. Hematoxylin and eosin. ×1000. (*below*). Liver of senile mouse—811-day-old female. The larger nuclei in old animals often contain intranuclear inclusions. Two conspicuous ones are seen here. We believe that such inclusions develop from individual nucleoli. Hematoxylin and eosin. ×1000.

brosis'. Again, our own studies concern the pancreas in Wistar Institute rats, in man and, in a preliminary way, in the C57 Black Mouse. The aspect of this gland is a variable one in our material. The presence of change is consistent in old age but the degree and even the type are different. For example, the striking alteration in the Wistar Institute rats is the formation of numerous spheroidal cavities within the parenchyma. These are lined by simple squamous, keratinizing epithelium. They often merge with one another to form very large spaces, destroying much of the secreting parenchyma, both exocrine and endocrine, of the pancreas. In the human and to date in the mouse material the initial stages of such cavity formation, namely, a proliferation of intralobular ducts or possibly of undifferentiated cells is seen but seems seldom to proceed to a degree comparable to that found in the rat. Metaplastic change in the interlobular ducts, a transformation of the simple columnar epithelium to one of stratified squamous variety, has been described (8) and we have found it in the majority of our senile specimens, with an occasional occurrence in the younger age group. It seems to us to fit in very well as a part of the tendency of the duct cells to proliferate in old age.

The pancreas shows a certain amount of adipose tissue replacement in senility which may be related to the hyperplastic processes described or which may occur independently of them.

Two other large glands in which we have studied the process of senescence are the salivary glands, the parotid and submandibular glands. Our investigations on these have been confined to the Wistar Institute rat and man (9, 10).

In the parotid gland of the rat and in both the parotid and submandibular glands of the human subject the processes of destruction of parenchyma and of replacement of it by relatively 'inert' tissue are far more conspicuous than in the liver and pancreas (fig. 2). Among the 54 senile rats studied, over half of them showed 'marked fatty degeneration' of the parotid gland, by which an involvement of 50% or more of the gland is indicated. Surprisingly, the submandibular glands of the rat show practically none of this process, while in both types of salivary glands in man it is conspicuous, although more intense in the parotid gland.

It should be emphasized that this process, as seen very clearly in the rat, is one of actual fatty degeneration of secreting cells rather than of mere substitution.

We are able to confirm the findings of Hamperl (11–13) concerning the presence of peculiar large cells, 'oncocytes' in senile salivary glands (figs. 3 and 4), and to show the process of amitotic division of the nucleus in such cells, which leads to the binucleate condition and probably to a formation of new cells.

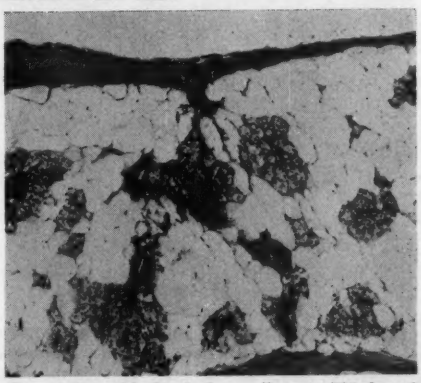


FIG. 2. Parotid gland of a senile rat—700-day-old female. Marked fatty degeneration has occurred. Hematoxylin and eosin. ×92.

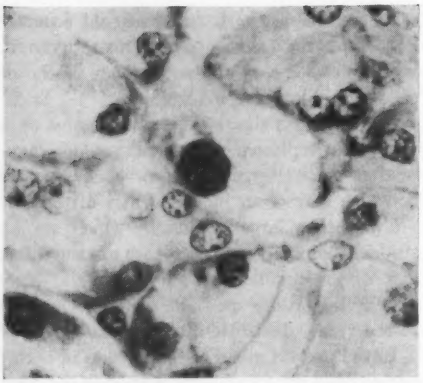


FIG. 3. Peculiar large cell, or oncocyte, in the parotid gland of a senile rat—700-day-old female. Note the appearance of the nucleus as compared to those of the 'normal' cells in the field. Hematoxylin and eosin. ×845.

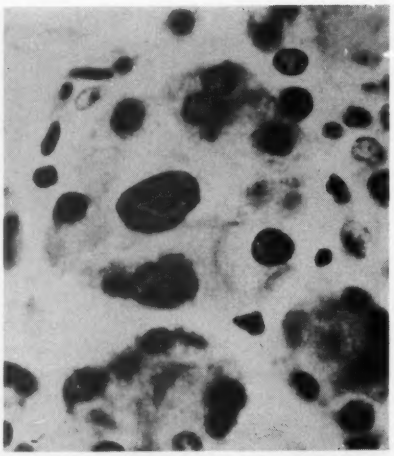


FIG. 4. Two oncocytes, one with a very large nucleus, in an alveolus of the parotid gland of a 1000-day-old male rat. Hematoxylin and eosin. ×900.

Other changes in salivary glands include lymphocytic infiltration in the interlobular spaces similar to that in the liver and, in many cases, the appearance of small calculi in the ducts, more commonly in our rat material. Fibrosis is seen in rare instances.

Recently we have completed a study on the kidneys of Wistar Institute rats (14). Interesting changes, including a hypertrophy of many glomeruli, are seen. Deposition of precipitates in the tubules is common. Lymphocytic infiltration is widespread. Again, however, there is little actual fibrosis.

We well may approach the subject of age changes in the endocrine glands with a feeling of anticipation and indeed of some excitement. It is here, in the controlling and regulating organs, that we might hope perhaps to see changes of major significance for the entire economy of the body and changes which might be amenable to experimental control through treatment with extracts or purified hormonal products or possibly by procedures of transplantation.

Even in this field the literature on changes due to age per se is remarkably scanty and some of it is conflicting. The thyroid gland, the great controller of the rate of metabolism, has been described by some authors (15, 16) as undergoing a regressive change with age in man, with fibrosis 'relatively more common' in old age.

On the other hand, Dogliotti and Nizzi-Nuti

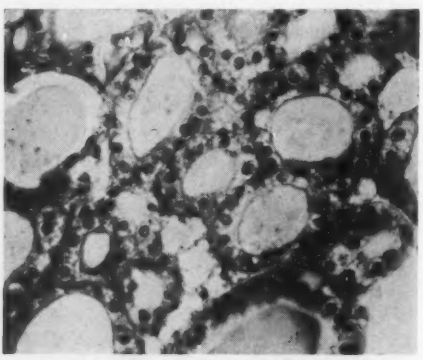


FIG. 5. Thyroid gland of a young mouse—213-day-old female. There is little connective tissue and the follicular epithelium is of simple cuboidal type. Hematoxylin and eosin. ×315.

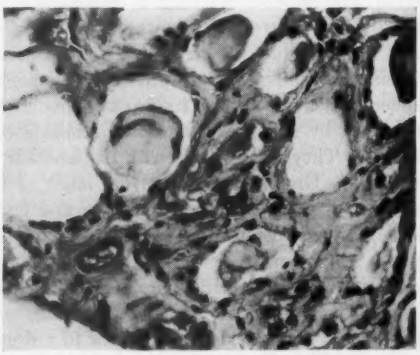


FIG. 6. Thyroid gland of a senile mouse—811-day-old female. Fibrous connective tissue is abundant. The epithelium in many follicles is of simple squamous type and the colloid often appears very dense. Hematoxylin and eosin ×315.

(17) believe that decreased colloid and increase in volume of epithelial cells in the thyroid glands of senile human subjects represent evidences of increased activity of the gland. Garau (18) believes that when such increased activity occurs it is compensatory to degeneration of follicles in other parts of the gland which are being 'submerged' by growth of fibrous connective tissue.

Hinton (19), after a study of 80 human subjects, decided that he was unable to draw conclusions as to 'what is normal thyroid tissue for the different ages'.

In the attempt to study general trends with age it has seemed well to us to turn from the more complicated problem in man to the somewhat less complicated one of the laboratory animal. Here, in an earlier study on the C57 Black mouse we found marked senile change in the thyroid gland (20). In current studies on our new colony of these animals we have confirmed and extended these findings (figs. 5 and 6). It is in the thyroid gland that we have actually the closest approach to what we might call a 'classical picture' of senile fibrosis. The scanty, chiefly reticular connective tissue of young and middle-aged mice is replaced in senility by abundant collagenous connective tissue. The individual follicles in old age show a surprising variety both in quantity and appearance of the contained colloid. The whole aspect of the gland in old age is one of atrophy of parenchyma and 'overgrowth' of connective tissue. Here is one strain of one laboratory animal in which experiments on the endocrine balance of the organism might well be undertaken on the basis of the obvious senile deterioration of the gland.

The literature on the pituitary and adrenal glands in old age in the rat (21, 22) shows a tendency to thickening of reticular fibers and some increase in number of collagenous fibers with advancing age, but a correlation with changes in the parenchyma here is not clear. Evidence of cell loss is not described, except in the cytological studies by Payne (23) on the endocrine glands of the fowl.

The nervous system is that portion of the organism which contains the most 'stable' elements of all, the nerve cells. It has been a subject of numerous studies by workers in many countries, yet today a definite conflict of views exists concerning not only the degree of change found here in old age but even as to whether really significant microscopic changes are seen! As a result of our personal investigations and our study of the literature, we feel that the uncertainty here is due chiefly to a lack of integration of much well-based work. Our own research has tended to confirm the findings of the majority of earlier workers in showing very clear-cut changes

FIG. 7. Giant cell of Betz from the motor cortex of a 29-year-old man. The Nissl substance is abundant and the karyoplasm is clear. Cresyl violet. ×1330

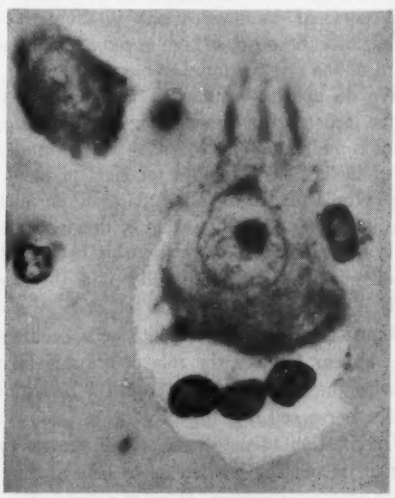


FIG. 8. Giant cell of Betz from the motor cortex of a 78-year-old woman. Nissl substance is scanty and the karyoplasm is lightly basophilic. The satellite cell to the right of the nerve cell contains granules of lipofuscin pigment. Cresyl violet. X1330.

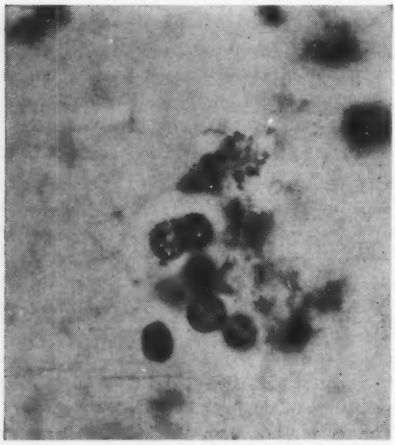


FIG 9. Nerve cell from the motor cortex of a 78-year-old woman in an advanced state of degeneration. The clustered satellite cells appear to be engaged in the removal of the neuronal debris. Cresyl violet. ×1330.

in the general aspect of nervous tissue in senescence if specific portions of the nervous system are compared with one another (24). We do not find that the total population of nerve cells survives to extreme old age of the organism but that degenerative changes lead on to actual death of many cells. The degree of cell loss, as seen in our animal material and by Brody (25 and personal communication) in human material, appears to run as high in some regions as a third of the original number present!

We do not have sufficient time here to describe in detail the types of degenerative change seen in individual nerve cells (figs. 7 and 8). They include accumulation of inert pigment in the cytoplasm; loss of Nissl material; shrinkage of the cell body; deformation of dendrites; and fatty degeneration. The relationship between the small glial cells (satellite cells) and the nerve cells is of interest. About the cell body of a degenerating neuron the satellite cells proliferate and appear to play the role of scavengers, removing the debris resulting from the breakdown of the nerve cell (fig. 9).

Observation of many regions of the nervous system in old age has led us to believe that in some groups of neurons there is a type of reactive change, a compensatory or defensive alteration designed to ward off the effects of aging. Such neurons show division of nucleoli or nuclei

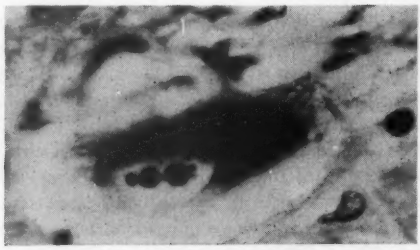


FIG. 10. Nerve cell from the substantia nigra of a 78-year-old woman, showing three nucleoli. Division of nucleoli appears to be a *reactive* type of change. Cresyl violet. ×1150.

with a retention of a youthful aspect of the cytoplasm.

Professor Oskar Vogt, Director of the Institute for Brain Research at Neustadt in the Black Forest, and his disciples have demonstrated such changes clearly in the hypothalamus where the maintenance of functions such as fluid balance and temperature control are of great importance to the organism. We have found the same phenomena in the substantia nigra in man (fig. 10) and among the Purkinje cells of the cerebellum in lower animals and agree with Professor Vogt's interpretation of its significance (24). In these locations, however, it is perhaps not as easy to see an urgent need for maintenance of function.

It would be of much interest to extend this discussion to the muscular and skeletal tissue, to the heart, and to the blood vessels. With these we have had less to do in our personal studies and we shall not attempt here to discuss the findings of others concerning them.

The last subject, therefore, to which we shall turn our attention is the problem of senile change in covering and lining layers of the body. Such layers consist of epithelium, the cells of which are being constantly lost, and of connective tissue. We have studied the skin, composed of the epidermis as the epithelial layer and the dermis as the connective tissue layer (26); and the mucosa of the small intestine, with its simple columnar epithelium and its tunica propria of connective tissue (27).

Age changes in a constantly renewed cell population might not be anticipated. In the intestinal epithelium, indeed, we have not seen evidences of change. In the epidermis of the rat, however, we have described a greater degree of adherence of lamellae of the stratum corneum and a marked

change in aspect of the stratum germinativum, with nuclei relatively smaller and more prominent cell boundaries and intercellular bridges in the senile specimens.

Changes in the dermis, particularly degeneration of elastic fibers, have been described (28). In our animal material this type of change was not seen. Unexpectedly, however, a fibrosis of the tunica propria of the intestine is a prominent feature in senile animals.

SUMMARY AND CONCLUSIONS

What conclusions can we draw from the rather varied data which we have presented? We feel that two general statements can be made: *1*) the description of 'senile fibrosis' as a characteristic picture of many organs in old age is certainly an over-simplification, although fibrotic change in varying degree does occur; *2*) the 'primary' change, so far as the visible histological alterations are concerned, seems to be in the parenchymal cells.

A number of the organs which we have disdussed show little or no fibrotic change even at a very advanced age. On the other hand, all of the organs do show alteration of the cells of the parenchyma. In some cases there is a clear-cut degeneration of these cells which seems to be the cause of the accumulation of inert tissue, as in the formation of adipose tissue which takes the place of parenchyma in the parotid gland. In other cases, even where fibrosis is prominent, as in the senile thyroid gland, large areas of the sections show changes in the follicles without fibrotic change having begun.

It would seem, then, that on the functional and physiological side our efforts should be directed to learning more about the changes in the essential cells of the various organs, for it is probable that the accumulations of fibrous and adipose tissue are only secondary phenomena.

The oft-quoted statement that 'cells as such are potentially immortal' might well receive a closer scrutiny. True, certain types of cells have been cultivated *in vitro* over long periods of time, but is it not the case that these have been cells of a relatively undifferentiated type, particularly the fibroblasts, or even cells of abnormal type, which would grow in the body as tumors? Do we really know whether the well-differentiated types of cells such as those of the glands and of the nervous system, are not limited by some inborn characteristics which may determine their span of life?

While we may look, then, to the essential cells of the major organs for more information on senescence, the question is still unresolved as to whether the process is one which is, as it were, 'fulfilling the destiny,' the genic potentiality of the cell types involved, or whether subtle changes in the environment are responsible for the senescence of the cell. We feel that it has been shown with a fair degree of certitude that the grosser changes of fibrosis and adipose tissue invasion are secondary rather than primary phenomena of senescence.

REFERENCES

1. Metchnikov, E. *The Prolongation of Life*, New York: Putnam, 1908.
2. Andrew, W., H. M. Brown and J. B. Johnson. *Am. J. Anat.*, 72: 199, 1943.
3. Wilson, J. W. *Anat. Rec.* 118 (2): 368, 1954.
4. Findlay, G. M. *Brit. J. Exp. Biology* 13: 223, 1932.
5. Wallbach, G. *Ztschr. ges. exper. Med.* 68: 569, 1929.
6. Korenchevsky, V., S. K. Paris and B. Benjamin. *J. Gerontol.* 5: 120, 1950.
7. Andrew, W. *Am. J. Anat.* 74: 97, 1944.
8. Baló, J. and H. C. Balon. *Arch. path.* 7: 27, 1929.
9. Andrew, W. *Am. J. Anat.* 85: 157, 1949.
10. Andrew, W. *J. Gerontol.* 7: 178, 1952.
11. Hamperl, H. *Ztschr. f. mikr. anat. Forsch.* 27: 1, 1931.
12. Hamperl, H. *Virchows Arch. path. Anat.* 291: 704, 1933.
13. Hamperl, *H. Virchows Arch. path. Anat.* 298: 327, 1937.
14. Andrew, W. *J. Gerontol.* 10: 466, 1955.
15. Nolan, L. E. *Arch. Path.* 25: 1, 1938.
16. Hertzler, A. E. *Arch. Surg.* 38: 417, 1939.
17. Dogliotti, C. G. and G. Nizzi-Nuti. *Endocrinology* 19: 289, 1935.
18. Garau, B. *Endocrinol. e Pat. Costit.* 13: 103, 1938.
19. Hinton, J. W. *Am. J. Surg.* 11: 269, 1931.
20. Andrew, W. and N. V. Andrew. *Am. J. Path.* 18: 849, 1942.
21. Lansing, W. and J. M. Wolfe. *Anat. Rec.* 83: 355, 1942.
22. Dribben, I. S. and J. M. Wolfe. *Anat. Rec.* 98: 557, 1947.
23. Payne, F. *J. Gerontol.* 4: 193, 1949.
24. Andrew, W. *J. Chronic Dis.* 3: 575, 1956.
25. Brody, H. *J. Comp. Neurol.* 102: 511, 1955.
26. Andrew, W. *Am. J. Anat.* 89: 283, 1951.
27. Andrew, W. *J. Gerontol.* (In press).
28. Ma, C. K. and E. V. Cowdry. *J. Gerontol.* 5: 203, 1950.

CELLULAR PHYSIOLOGY AND AGING

L. V. Heilbrunn

From the Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania

The aging of an animal is undoubtedly due to the aging of the cells of which it is composed. And this aging can occur in all types of cells. Cytologists have been able to describe changes in the appearance of cells as they become old. But the meaning of these morphological changes and their relation to the aging process is not very clear.

The cell is a colloidal engine. What we would like to know is how this engine changes as it begins to wear out.

When cells are studied from the standpoint of colloid chemistry, it can be shown that they consist of an outer firm region or cortex, inside of which is the main mass of the fluid protoplasm. This type of basic structure is certainly to be found in protozoan cells, in marine egg cells, in muscle cells and in nerve cells. The width of the cortex varies rather widely. In the unfertilized sea urchin egg it is only about a micron or two thick. In frog muscle fibers, the cortex is approximately 10 microns thick. There is an increasing amount of evidence which indicates that the cortex is to a large extent responsible for the inability of many substances to penetrate into the cell interior. This does not mean that there are no other membranes either just outside or just inside of the cortex which may not also play a part in determining the semipermeability of what we know physiologically as the plasma membrane of the cell.

The rigidity of the cell cortex and the permeability of the cell both depend on the presence of calcium. Remove calcium from the cortex and to a large extent it loses its rigidity. Moreover as calcium is removed or replaced by monovalent ions, the permeability of the cell increases.

Indeed, more than any other naturally occurring cation, calcium influences the colloids of the cell. Thus when protoplasm changes from a fluid condition to a more or less solid state, the primary reaction involved is a clotting reaction which in its early stages requires the presence of free calcium. The evidence for this statement is voluminous (1, 2).

Because of the importance of calcium for the colloidal reactions of living protoplasm, it is well to inquire if the protoplasmic colloid as it ages does not show differences in its relationship to the calcium ion. Evidence from chemical studies favors this view. Many authors have shown that in aging tissues the calcium increases progressively, and although the results of such studies are not completely concordant, the indication is strong that as organs and tissues grow older, their calcium content increases (3). On the other hand in old organs and tissues the potassium content goes down (4). Of course in interpreting the data on whole organs and tissues it must be remembered that any change in chemical constitution may depend not so much on a change in the chemistry of the protoplasm, but rather on a change in the non-cellular materials, such as connective tissue fibers.

If it is true that the calcium content of cells increases with age, then we are immediately led to inquire as to why this should happen. We might assume that the calcium binding power of the cell proteins changes as the cells grow older. This is a logical assumption and it is supported by some interesting experiments of Lansing, Rosenthal and Kamen (5). These workers injected into the peritoneal cavities of mice a solution of calcium chloride which contained 1 mg calcium/cc and enough radioactive calcium (Ca^{45}) to give 50,000 counts per minute. Lansing, Rosenthal and Kamen had a total of 10 mice, 5 of these were 2–3 months old, the other 5 were somewhat older, that is to say they were 12–13 months old and full grown. At varying times after the injection of the calcium chloride into the peritoneum, the mice were killed and their livers were analyzed both for free and for bound calcium. Then it could be shown (see fig. 1) that in the livers of the older mice there was markedly more calcium, also that in the livers of the older mice the amount of bound calcium as compared to the amount of free calcium was somewhat greater. (Bound calcium was separated from free calcium by ultrafiltration). From these experi-

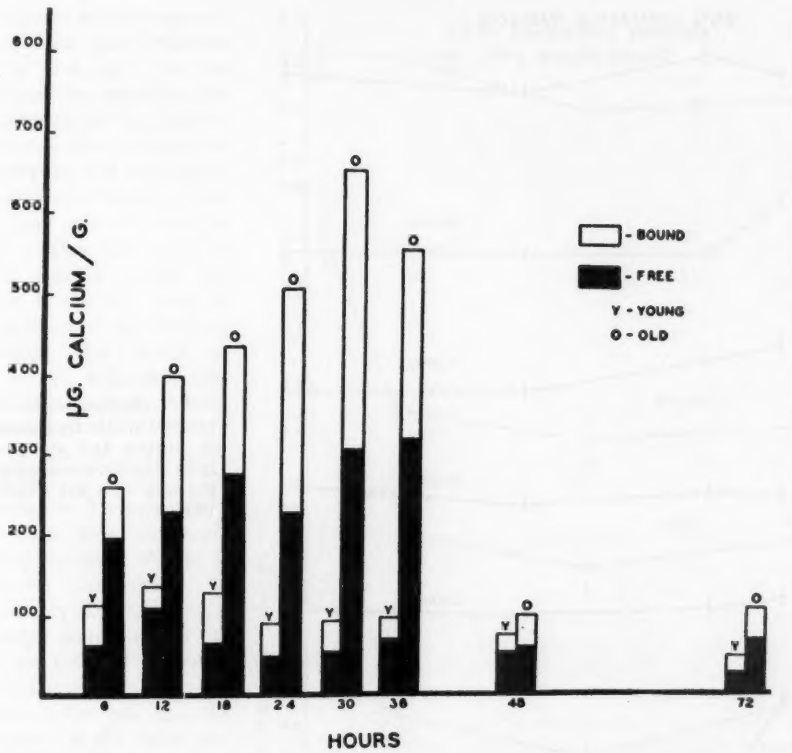


FIG. 1. The relation between free and bound calcium of young and old mouse liver as a function of time after injection of calcium (Lansing, Rosenthal and Kamen).

ments the authors concluded that in young animals calcium uptake by tissues is low whereas in older animals it is much more rapid. We might assume therefore that in older animals the proteins or lipoproteins of the tissues have a greater affinity for calcium than do similar compounds in the tissues of younger animals.

Impressed with the significance of these results, I asked a student of mine, Mr. Harry Weller (now Dr. Weller), to continue this type of work on the tissues of rats. Weller studied the calcium binding of homogenates of various organs of rats at different ages (6). He used four groups of 10 rats each; rats of the youngest group were 30–37 days old, those of the next older group were 5–5½ months old, the third group included rats 14–15 months old, and the oldest group consisted of rats 26–28 months old. Weller studied not only the liver, but the heart, psoas muscle, brain, kidney and skin as well. The various tissues and organs were homogenized in 0.005 M calcium chloride and the calcium uptake was determined by equilibrium dialysis. The homogenates were placed in cellophane sacs and stored overnight in a refrigerator at about 5° C. As the material in the interior of the sacs takes up calcium the calcium concentration of the solution surrounding the sac will drop so as to reestablish equilibrium.

Weller's results are shown in figures 2 and 3. It will be seen at a glance that in senescent rats there is no significant increase in the avidity of homogenized tissues for calcium.

There is then an apparent conflict between the results obtained by Weller and those obtained by Lansing, Rosenthal and Kamen. How can we explain this, or is indeed any explanation possible?

In the first place, it must be remembered that whereas Lansing and his collaborators studied the uptake of calcium by intact cells, Weller was interested only in homogenates. Weller's work indicates clearly that the colloids of the proto-

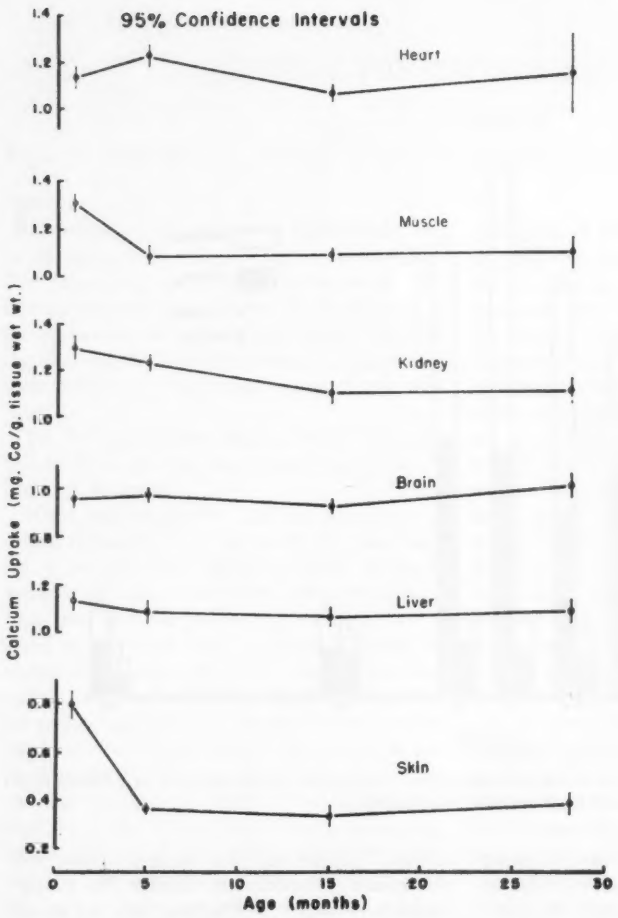


FIG. 2. Comparison of the calcium uptake by homogenates of organs and tissues taken from rats of various ages. Comparisons on a wet weight basis (Weller).

plasm as a whole show little change in their avidity for calcium. But might it not be plausible to assume that even though the colloids in the interior of the cell do not change in their power to bind calcium, the availability of calcium for these proteins might become greater in advancing age? In other words it is possible that in older cells calcium can enter the protoplasm more freely. In what follows an effort will be made to present evidence in support of this point of view.

Let us consider again the nature of the cell as a colloidal system. Typically, the fluid colloid in the interior of the cell is surrounded by a rigid cortex, and this cortex may be regarded as the chief obstacle to the passage of ions into the interior. Thus Mazia (7) pictures the cortex as constituting both a reservoir of ions and a barrier to their entrance into the cell interior. This is especially true of cations, which are known to be bound by the colloids of the cortex. It would be interesting if we could establish the fact that as a cell ages, the cortex changes in such a way as to offer less resistance to the entrance of calcium into the cell interior. If this were true, then we could explain the apparent conflict between the results of Weller and those of Lansing, Rosenthal and Kamen.

Does the cortex change its properties with age? As yet there is no evidence whatsoever for the cortex of the tissue cells of higher animals. Indeed, at the present time the only types of cells in which useful physical studies of the cortex

can be made are protozoa and marine eggs. And protozoan cells do not age. On the other hand the eggs of marine invertebrates age very rapidly, but there is always a question as to whether this aging process is basically similar to the aging that occurs in brain or muscle or liver. And yet, if in marine eggs we could find changes in the cell cortex of the exact nature that we have postulated, this would at least be an indication that our theory is worth further consideration.

For a number of summers, Dr. Walter L. Wilson of the University of Vermont and I have been now and again studying the aging of eggs of the worm Chaetopterus. In the Chaetopterus egg it is a relatively simple matter to study the colloidal properties both of the protoplasm in the interior of the cell and the protoplasm in the outer cortex. The interior protoplasm is very fluid and only weak centrifugal forces are sufficient to move the granules through the protoplasm in a matter of a few seconds. However the cortex is rigid. In order to move granules out of the cortex, a force of about 6000 times gravity is necessary.

The rigidity of the cortex can be measured quantitatively by determining the amount of centrifugal force necessary to dislodge from it most of the granules it normally contains. In the course of other studies, we noticed that the rigidity of the cell cortex in Chaetopterus eggs keeps decreasing with time. Accordingly we determined to follow this progressive decrease in rigidity as accurately as possible.

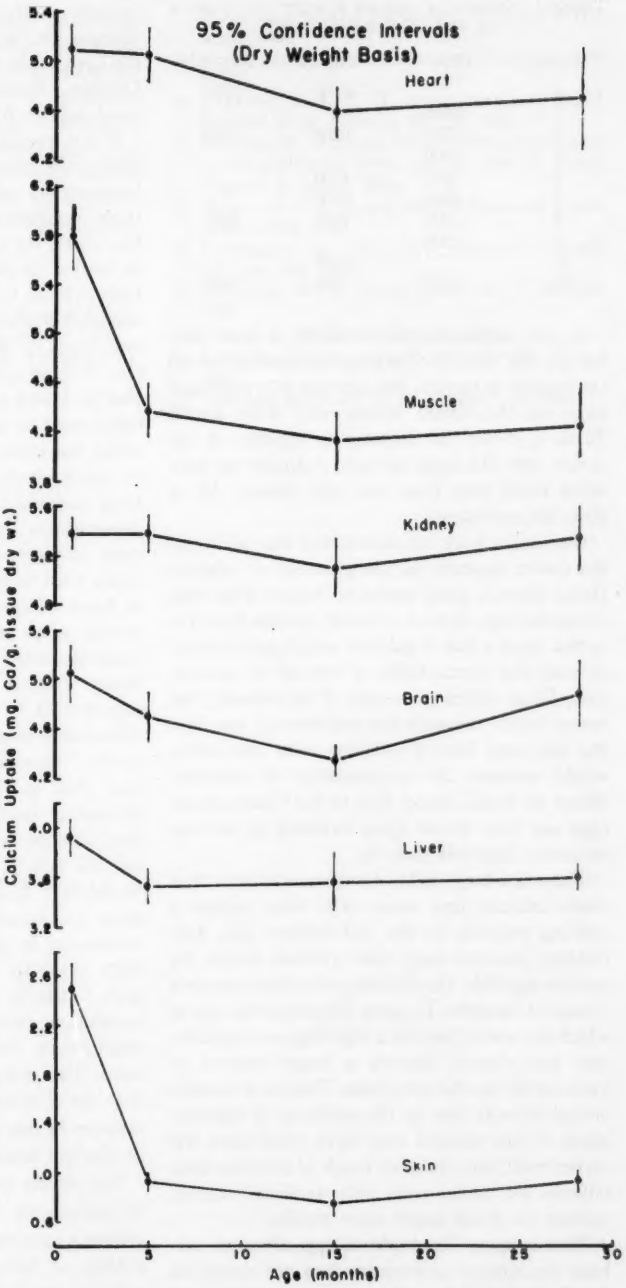


FIG. 3. Comparisons of the calcium uptake by homogenates or organs and tissues taken from rats of various ages. Comparisons on a dry weight basis (Weller).

TABLE 1. EFFECT OF AGE ON RIGIDITY OF CORTEX OF CHAETOPTERUS EGGS

Hours	Exp. A	Exp. B	Exp. C
½		6100	6100
1	6900		
2	4700	5200	5000
3	3900		
4	3500	4700	
5	3000	4300	
6	2500	4100	3900
7	2200		
10		4100	
24	1200	1500	1000

In our measurements of rigidity a hand centrifuge was used. By varying the speed at which the handle is turned, the amount of centrifugal force can be varied within very wide limits. Table 1 shows the decrease in rigidity of the cortex with the lapse of time. Actually we have much more data than the table shows. All of them are consistent.

Our earlier work has shown that the rigidity of the cortex depends on the presence of calcium. Hence there is good reason to believe that with advancing age, there is a loss of calcium from the cortex. Such a loss of calcium would undoubtedly increase the permeability of the cell to calcium ions, for as noted previously it is primarily the cortex which prevents the entrance of ions into the cell, and loss of calcium from the cortex would increase its permeability to calcium. Hence we would expect that as the Chaetopterus eggs age they would show evidence of calcium entrance. And this they do.

There is a large body of evidence to show that when calcium ions enter cells they induce a clotting reaction in the cell interior (2). This clotting reaction may take various forms. In marine egg cells, the clotted protoplasm becomes a mass of vacuoles. In aging Chaetopterus eggs in which the cortex has lost a high degree of rigidity, one can always observe a large number of vacuoles within the cytoplasm. This vacuolization is undoubtedly due to the entrance of calcium. Some of this calcium may have come from the cortex itself, but probably much of it comes from outside, for in the cells with weakened cortex, calcium no doubt enters more rapidly.

Thus in aging Chaetopterus eggs, although perhaps the interior protoplasm does not change in its affinity for calcium, the cortex does change and change in such a way as to facilitate the entrance of calcium into the cell interior. And once this calcium enters it may produce destructive changes. On the basis of this work we can explain the apparently contradictory results obtained by Lansing, Rosenthal and Kamen on the one hand and by Weller on the other.

If it is true that in aging animals calcium enters tissue cells more readily, then there would be a tendency for calcium to leave the blood. Under these conditions, calcium would be sucked out of the bones, for when the blood shows a tendency to be low in calcium, the bones yield some of their calcium to it (8). The bones of old men and women are often low in calcium and, as a result, two types of disease are common in the aged. One of these is osteoporosis, a disease so common that a recent clinical author has called it the commonest of all diseases (9). The other disease, which has often been confused with osteoporosis, is osteomalacia. (In osteoporosis there is less bony material, i.e. more cavities in the bones; in osteomalacia there is a smaller amount of calcium throughout the bone. In osteoporosis, bones tend to break; in osteomalacia, they tend to bend). Cooke in 1955 (10) stated that osteoporosis is an extremely common condition and yet it is scarcely discussed at all in medical texts. Many authors have stressed the relation of osteoporosis to endocrine disturbances or to a disturbance in protein metabolism and this is the point of view taken in Cecil's *Textbook of Medicine*. But in recent years there has been an increasing amount of information to indicate that osteoporosis may be primarily related to calcium lack. Thus, for example, at the end of World War I, both in Austria and Germany, there was considerable evidence of osteoporosis developing in people who had calcium-deficient diets (11). In 1953, Vintner-Paulsen (12) reported that in old people there was a marked correlation between low calcium intake and osteoporosis. Other references could also be cited. The point to be emphasized here is that this loss of calcium from the bones may in large measure be due to an increased uptake of calcium by the soft tissues and organs.

Our results indicate that this increased uptake of calcium by the cells of various organs and tissues is apparently not due to any change in the avidity of the colloids in the cell interior for calcium, but that the cortex of the cell becomes weakened so as to allow more calcium to enter. This additional calcium can cause degenerative

changes. Of course additional work is needed, for we would like to know whether the tissue cells of higher animals do actually behave like the marine egg cells we have studied. And we should also like to know what factors might conceivably prevent the weakening of the cell cortex and thus prevent senescence. That such factors do exist is indicated by the fact that as long as the egg cells are retained within the body of the animal, their aging is greatly retarded.

REFERENCES

1. Heilbrunn, L. V. *An Outline of General Physiology* (3rd ed.). Philadelphia: Saunders, 1952.
2. Heilbrunn, L. V. *The Dynamics of Living Protoplasm*. New York: Acad. Press, 1956.
3. Lansing, A. I. (editor). In *Cowdry's Problems of Ageing* (3rd ed.). Baltimore: Williams & Wilkins. 1952.
4. Simms, H. S. and A. Stolman. *Science* 86: 269, 1937.
5. Lansing, A. I., T. B. Rosenthal and M. D. Kamen. *Arch. Biochem.* 20: 125, 1948.
6. Wfller, H. Doctoral Dissertation. University of Pennsylvania, 1955. Also *J. Cell. & Comp. Physiol.* In press, 1956.
7. Mazia, D. *Cold Spring Harbor Symp. on Quant. Biol.* 8: 195, 1940.
8. Taubmann, G. *Arch. exper. Path. u. Pharmakol.* 174: 476, 1934.
9. Jacobson, W. P. *South African M. J.* 29: 885, 1955.
10. Cooke, A. M. *Lancet* 268: 877, 1955.
11. Alwens. *Münch. med. Wochenschr.* 66: 1071, 1919.
12. Vintner-Paulsen, N. *Geriatrics* 8: 76, 1953.

CELLULAR METABOLISM AND AGING

CHARLES H. BARROWS, JR.

From the Gerontology Branch, National Heart Institute, National Institutes of Health and the Baltimore City Hospitals, Baltimore, Maryland

IT IS LOGICAL to assume that some alteration in physiological or metabolic function of individual cells must form the basis for the various changes which have been attributed to increasing age. It is the aim of this discussion to review briefly studies which have attempted to identify age changes in cellular metabolism and to point out certain basic difficulties which limit interpretation of the results. Probably the greatest of these difficulties lies in the fact that many investigators have used young animals which were still in the growth phase. Under these circumstances, it is impossible to distinguish between growth changes and changes which may be attributed to senescence. Another major problem is the basis on which the measured enzyme activities were calculated. Among those used have been the wet weight, dry weight, nitrogen and protein nitrogen content, the DNA content, as well as an actual cell count of the tissues. To further complicate comparisons, some investigators have used tissue slices, whereas others have used tissue homogenates. Finally, different investigators have studied different species of animals as well as different tissues. Since we do not know the extent to which senescence is a common property of all species and all types of cells, we cannot conclude that differences observed can be attributed to age.

Total Oxygen Uptake—Tissue Slices. Total oxygen uptake of tissue slices or homogenates has been used as an index of overall metabolic activity. Many of these studies have been based on tissue slices of a variety of organs. In table 1, the data of Wollenberger and Jehl (1) demonstrate that the steady level of respiration, based on the dry weight of guinea pig ventricular slices, decreased from birth until the age of 5–6 months, at which time growth is nearly completed. No further change in the respiration of ventricular tissue slices was observed in the older animals. The data of Pearce (2) also demonstrate a decreased rate of oxygen uptake, per unit wet weight of mouse ventricular tissue with age, when 4 to 9-week-old mice were compared to 50 to 60-week-old animals. Similar findings were observed by Pearce when kidney and liver slices were examined. It should be pointed out that the young animals used in this latter study may be considered well within the growth phase. A number of investigators have examined the effect of age on the oxygen uptake of rat liver slices. These data are presented in table 2. Jacob (3) observed a decreased oxygen uptake of female rat liver slices over the age span of 30 days to 2 years when the activity was based on the wet weight of the tissues. (The age of the adult animals was not given.) The data of Ross and Ely (4) indicate a similar decrement with age when the oxygen uptake of liver slices of male rats was calculated on the basis of wet weight, dry weight or the nitrogen content of the tissue (table 2). Since the 21-month-old rats were appreciably heavier than the 5- to 6-month-old rats, we may assume that the younger animals were still actively growing. However, Hawkins (5) failed to find any effect of age on the oxygen uptake of rat liver slices when the activity was based on the dry weight of the tissue even during the growth period. Finally, Rosenthal (6) demonstrated a decreased oxygen uptake per unit dry weight of bovine articular cartilage. Therefore, with the exception of the data obtained by Hawkins, these data are consistent in showing a difference between the young growing animals and the senescent animal, with respect to the oxygen uptake of the various tissues. However, no change, which may be attributed to senescence, is discernible when adult animals are compared to the senescent ones. Furthermore, these data, in the form originally presented, fail to answer the question as to the effect of senescence on the metabolic activity of cells, since the bases on which the activity was calculated may not serve as reliable indices of the number of existing cells in tissues. For example, the agewise increased deposition of acellular elements such as collagen and fibrin, which contributes to the dry weight and protein nitrogen of the tissue, may mask the loss of cells. Jacob

TABLE 1. RESPIRATION OF TISSUE SLICES AND AGE

Investigator	Age	Guinea Pig Heart μl. O_2/hr/mg dry wt.		
Wollenberger & Jehl (1)	<1 d.	4.1±0.18		
	10 d.	3.6±0.17		
	19–24 d.	3.3±0.17		
	5–6 mo.	2.5±0.17		
	30 mo.	1.9±0.18		
	5–6 yr.	2.3±0.19		
		Mouse Tissues μl. O_2/min/gm wet wt.		
		Heart	Liver	Kidney
Pearce (2)	4–9 wk.	14.68±0.21	16.14±0.24	50.10±0.55
	50–60 wk.	11.46±0.11	11.97±0.32	44.96±0.55

TABLE 2. RESPIRATION OF TISSUE SLICES AND AGE

Age	Weight gm.	μl. O_2/mg/hr.	μl. $O_2 \times 10^{-5}$/ nucleus/hr.	μl. $O_2 \times 10^{-5}$/ cell/hr.*
		Rat Liver—Jacob (3)		
30 d.	66	1.82±0.31	0.74	1.18
60 d.	129	1.40±0.30	0.85	
adult	219	1.22±0.25	1.04	1.36
2 yr.	243	0.80±0.22	0.60	0.81
		Rat Liver—Ross & Ely (4)		
		μl. O_2/mg wet wt/hr.		μl. $O_2 \times 10^{-5}$/ cell/hr.*
1 mo.	40	1.51±0.04		0.66
5–6 mo.	165	1.20±0.03		0.81
21 mo.	475	1.03±0.03		0.65
		Rat Liver—Hawkins (5)		
		μl. O_2/mg dry wt/hr.		
3–21 d.		11.5		
12 mo.		9.4		
22 mo.		13.2		
		Bovine Articular Cartilage—Rosenthal et al. (6)		
		μl. O_2/mg dry wt/hr.		μl. $O_2 \times 10^{-5}$/ cell/hr.
< ½ y.		0.088±0.017		0.66
1–7 yr.		0.024±0.010		0.51
8–11 yr.		0.008±0.005		0.24

* Calculated from mean values.

(table 2) determined the DNA content of the tissue as well as the DNA per nucleus of liver tissue and demonstrated an increase with age in the oxygen uptake, per nucleus, up to the adult, and a striking decrease between the adult and the two-year-old animal. Since the number of binucleated liver cells varies with age, these data do not, in themselves, represent the oxygen uptake per cell. If the vaules of binucleated cells, reported by St. Aubin (7), are applied, and if the adults were at least five months old, which their body weight indicates, then the calculated oxygen uptake per liver cell decreases strikingly in the senescent rat, as shown in the last column of table 2. In contrast, Ross and Ely determined the number of nuclei per gram of liver by counting nuclei in citric acid homogenates. It is possible to make a rough estimate of the oxygen uptake, per liver cell, assuming that fragmentation of nuclei during preparation was similar in the old and growing tissue, and adjusting the values for binucleate cells according to St. Aubin. These calculations show no apparent agewise decrease in the oygen uptake per liver cell. Rosenthal *et al.* reported a decreased oxygen uptake of bovine articular cartilage with age when the activity was based on the actual cell count of the tissues. We are thus faced with conflicting evidence with regard to changes in oxygen uptake in senescence. Additional data are needed on different species and tissues before definitive conclusions can be reached.

Total Oxygen Uptake in Tissue Homogenates. In addition to these studies employing slices, the overall endogenous oxygen uptake has been determined in tissue homogenates of young and old animals. For example, Rafsky and Newman (8) determined the oxygen consumption of liver and kidney homogenates of 8-week, 50-week and 100-week-old guinea pigs. These data, as shown in table 3, demonstrate a marked reduction in the oxygen uptake of kidney, but not liver, homogenates over the age span of 8–100 weeks, based on either dry weight (table 3) or total nitrogen. The differences between middle-aged and old animals were significant for kidney, but not liver, homogenates. Reiner (9), employing rat liver homogenates fortified with various cofactors such as ATP, DPN, cytochrome *c*, as well as glucose, found no systematic decrease in respiration over the age span of 2–36 months. The respiration of brain homogenates was essentially constant from birth to 24 months, after which a sharp decline occurred. Although the results obtained on kidney homogenates demonstrate the same reduced activity as do the data of Pearce, who employed kidney slices (table 1), data on liver homogenates showed a much smaller decrement. The discrepancies between observations on tissue slices and homogenates may be attributed to the maintenance of cellular organization and presumably physiological concentrations of various tissue components in the slices, whereas the homogenates lack organization and the tissue components are considerably diluted.

The anaerobic glycolysis of rat liver slices has been examined by Ross and Ely as well as Haw-

TABLE 3. RESPIRATION OF TISSUE HOMOGENATES AND AGE

Investigator	Age	Guinea Pig Tissues μl. O_2/mg dry wt/hr. Liver	Kidney	Brain
Rafsky (8)	8 wk.	1.14±0.05	4.06±0.90	
	50–52 wk.	1.11±0.10	1.42±0.14	
	100 wk.	0.97±0.07	1.15±0.07	
		Rat Tissues μl. O_2/mg dry wt/hr.		
Reiner (9)	0– 1 mo.	7±1.0		14±0.8
	2– 4 mo.	10±1.3		15±1.3
	4– 6 mo.	12±3.0		15±2.5
	6–12 mo.	11±1.6		16±2.0
	12–18 mo.	11±1.5		15±1.7
	18–24 mo.	10±1.1		15±2.3
	24 mo.	9±2.2		10±1.6

kins. The data summarized in table 4 indicate a substantial agewise decrease in this metabolic activity, when the activity was based on either the dry or wet weight of the tissue. On the other hand, Reiner failed to demonstrate such a decrease in fortified rat liver homogenates. Thus, the discrepancy between the data of experiments employing tissue slices and those employing tissue homogenates is again apparent. No agewise effect on anaerobic glycolysis was observed by Reiner when fortified rat brain homogenates were examined. A reduced anaerobic glycolysis of bovine articular cartilage was reported by Rosenthal *et al.* when the activity was based on the dry weight of the tissue, but no such difference occurred when the actual cell count of the tissue was considered. By applying the same technique, previously described, to estimate cell number to the data obtained by Ross, an apparent decrease in the anaerobic glycolysis of liver cells may be seen. The mutual incompatibility of the data obtained by Ross and Rosenthal, both in regard to endogenous oxygen uptake and anaerobic glycolysis of the individual cells, may be attributed to differences in species and tissues employed. However, the discrepancy between the data obtained by Ross and those of Jacob on endogenous oxygen uptake of liver cells may be attributed to the different techniques employed to estimate cell numbers.

Specific Enzyme Systems. This examination of experimental data on overall metabolism, as measured by total oxygen uptake or anaerobic glycolysis, does not offer striking evidence of age changes in individual cells. However, it is possible that specific enzyme systems may undergo alterations in senescence. In view of the complex nature of cellular metabolism, it is difficult to decide which systems may be identified

TABLE 4. EFFECT OF AGE ON ANAEROBIC GLYCOLYSIS OF TISSUES

Age	μl. CO_2/mg wet wt/hr.	μl. $CO_2 \times 10^{-5}$/cell/hr.*
Rat Liver (slices)—Ross & Ely (4)		
1 mo.	1.24±0.06	0.54
5–6 mo.	0.86±0.07	0.58
21 mo.	0.57±0.07	0.36
Rat Liver (slices)—Hawkins (5)		
	μl. CO_2/mg dry wt/hr.	
3–21 d.	5.7	
12 mo.	2.0	
22 mo.	0.8	
Rat Tissue (homogenates)—Reiner (9)		
	μl. CO_2/mg dry wt/hr. Liver	Brain
0– 1 mo.	29±3.3	55±6.5
2– 4 mo.	19±2.3	76±4.5
4– 6 mo.	18±1.0	67±3.5
6–12 mo.	20±2.0	61±2.5
12–18 mo.	18±1.5	59±7.4
18–24 mo.	18±2.7	54±2.9
>24 mo.	19±2.6	60±5.1
Bovine Articular Cartilage (slices)—Rosenthal et al. (6)		
	μl. CO_2/mg dry wt/hr.	μl. $CO_2 \times 10^{-5}$/cell/hr.
<½ yr.	1.120±0.101	0.845
1–7 yr.	0.438±0.089	0.925
8–11 yr.	0.259±0.059	0.764

* Calculated from mean values.

with senescence. One criterion for choosing a specific system for study might well be the present status of biochemical knowledge. Another choice might be those systems which are closely concerned with energy production. In general, the enzymes which control the larger portion of the oxygen uptake of the tissue are an integral part of the Krebs citric acid cycle. This system may be especially significant as it is believed that the oxidation of its various intermediate products results in the formation of the high energy phosphate bonds of ATP. Put in its simplest form, the oxidation of these intermediates is their anaerobic dehydrogenation and the later combination of the hydrogen atoms with oxygen to form water. Ross and Ely have studied the anaerobic dehydrogenation, or dehydrogenase activity, of rat liver homogenates, and the data shown in table 5 demonstrate an increase in concentration of succinic dehydrogenase with age up to 6 months, but no change in older animals. This change existed when the activity was based on the wet weight, as given in table 4, as well as the dry weight or nitrogen content of the tissue.

TABLE 5. EFFECT OF AGE ON DEHYDROGENASE ACTIVITY OF TISSUES

	Age, months		
	1	5–6	21
Rat Liver—Ross & Ely (4)			
Activity*/mg wet wt.			
Succinic dehydrogenase	0.86	1.09	1.09
Malic dehydrogenase	16.22	17.10	19.12
Lactic dehydrogenase	0.41	0.66	0.73
Activity/cell $\times 10^{-7}$			
Succinic dehydrogenase	37.4	73.6	69.0
Malic dehydrogenase	7.1	11.6	12.1
Lactic dehydrogenase	17.9	42.5	46.0
Activity/mitochondrion $\times 10^{-14}$			
Succinic dehydrogenase	0.79	0.91	0.84
Malic dehydrogenase	14.9	14.2	14.8
Lactic dehydrogenase	0.37	0.55	0.56

Horse Heart—Lawrie (10)

	Age, years		
μl. O_2/mg dry fat-free wt/hr.	0.5	1.5	2–12
Succinic dehydrogenase	60	240	210
Cytochrome c oxidase	1500	2700	2800
Succinoxidase	160	580	610

* Formozan produced/mg/10 min. for succinic and lactic dehydrogenase; μl. O_2/mg/hr. for malic dehydrogenase.

Similarly, Lawrie (10) observed an increase in the concentration of succinic dehydrogenase of horse heart muscle, with age up to 1½ years, but no change in the activity occurred in older animals. In addition, the same changes appeared when the enzyme concerned with the second phase of the oxidation of succinate (i.e., cytochrome *c* oxidase) was examined. Furthermore, a system measuring the integration of these two enzymes, namely succinoxidase, was found to follow the pattern of changes observed in the two independent systems. Thus, these data fail to indicate any change in the concentration of the enzymes controlling the oxidation of succinate during senescence.

The continued increased activity with age of the lactic and malic dehydrogenase activity, as compared to the succinic dehydrogenase in the data of Ross, may indicate an age change in the former systems. However, the contrast between the increased activity of the various dehydrogenases, when the activities were based on either wet weight or cell count and the uniform activity based on mitochondria count, may raise a question as to whether these changes can be attributed to senescence. It is well known that certain physiologically active components reside almost, if not exclusively, in a given particulate of the cell. Since senescence may be associated with changes in the various particulates of the cell, it may be argued that if an enzyme is restricted to a given particulate, the activity per particulate should be determined rather than activity per cell.

Cross-Sectional Studies. A number of cross-sectional studies of enzyme activity in specific tissues have been made which give data on groups of animals at a number of points in their life span. For example, Dyrbye and Kirk (11) have determined the β-glucuronidase activity of human aortic and pulmonary arterial tissue in subjects ranging from birth to 88 years of age. These data, presented in table 6, demonstrate an increase in the activity of this enzyme, based on nitrogen content, or wet weight, from birth to 60–69 years of age in the case of aortic tissue and 50–59 in the case of pulmonary arterial tissue. The concentration of this enzyme in both tissues then decreased progressively throughout the latter age groups. In rats, this same pattern is evident in the work of Lightbody (12) who determined the arginase activity of liver tissue. The concentration of this enzyme increased to a peak value which was attained at 4 months of age, after which the activity decreased continuously to the 13–18 months of life. Data obtained by Zorzoli (13) on the acid phosphatase of mouse liver indicate a slight increase in concentration of the enzyme during the first 9 months of life, after which a striking decrease occurred.

All of the specific enzymes discussed above show a similar pattern of age changes; viz., a rise in concentration during growth with stable values during adulthood and a gradual decrement during senescence.

There are, however, some enzymes which decrease in concentration during growth; one example is alkaline phosphatase which diminishes in the plasma of humans (table 7 from Clark *et al.*, 14) and in mouse liver (table 7 from Zorzoli) up to adult life. During old age, there is a reversal, with a rise in enzyme concentration. Thus, during senescence, the direction of change is the reverse of that seen during growth. Although other enzymes, such as cathepsin (4), diminish during growth, we have no data on changes in this enzyme during senescence. This enzyme, along with others, should be investigated to see whether it also rises during senescence. If it is generally true that there is a group of enzymes whose pattern of age changes is opposite to that of the majority,

TABLE 6. EFFECT OF AGE ON VARIOUS ENZYMES

β-glucuronidase *Man—Dyrbye & Kirk (11)*			Arginase *Rat Liver—Lightbody (12)*		Acid Phosphatase *Mouse Liver—Zorzoli (13)*	
Age yr.	Aorta u/gm N	Pul. Artery u/gm N	Age mo.	μmol CO_2/mg dry wt.	Age mo.	γP/mg N
0–9	770		1	200.3±3.5	1	358
10–19	1003	1448	2	232.0±8.6	2	383
20–29	1220	1223	4	274.3±7.6	3	373
30–39	1390	1176	6–7	248.8±6.7	4	373
40–49	2230	1335	13–18	210.3±6.5	5	387
50–59	2390	3330			6	358
60–69	2790	3090			7	379
70–79	2190	2990			8	361
80–88	1745	2410			9	420
					11	400
					12	371
					17	322
					20	321
					24	288

TABLE 7. EFFECT OF AGE ON ALKALINE PHOSPHATASE OF TISSUES

Man (plasma)—Clark et al. (14)		Mouse (liver)—Zorzoli (13)	
Age yr.	NPN units	Age mo.	γP/mg wet wt.
1	7.6	1	3.17
2	6.6	2	3.74
3	6.3	3	3.51
12	4.7	4	3.62
18	2.4	5	3.65
26	1.8	6	3.35
32	1.6	7	3.58
43	1.7	8	3.14
55	2.5	9	3.25
83	2.8	11	2.95
88	2.9	12	2.77
		17	4.00
		20	4.33
		21	4.66
		24	4.48

investigations of the relationships between the two groups may provide information useful to the study of senescence.

In insects, concentrations of acid phosphatase, as well as cholinesterase, rise during growth and development, but do not change significantly in old age. Rockstein (15, 16) determined these enzymes in homogenates prepared from entire honeybees, as well as from the bee brain. During the first 8–10 days of life, the total body acid phosphatase and the total brain cholinesterase increased, but no change was apparent from the 10th day of life to very old age. The reverse relationship of alkaline phosphatase to other enzymes was also found in the honeybee. The total body alkaline phosphatase declined steadily during the first 7–10 days of life, after which no significant change was observed. This apparent lack of agreement with the other data presented may find its basis in the fact that the data of Rockstein represent changes in the total amount of enzymic activities rather than changes in their concentration. It is interesting that in the honeybee the total cholinesterase of brain tissue was unchanged even at old age, in spite of a loss of cells in two specific regions of the brain studied. This may be interpreted as demonstrating an increased cholinesterase per cell in the honeybee. This interpretation must be accepted with caution, since it is based on the assumption that the cellular loss observed in the specific sections of the brain examined is representative of the cellular loss in the total brain.

SUMMARY

There appears to be a rather good agreement among investigators that with advancing age there is a decrease in overall metabolic activity (total oxygen uptake and anaerobic glycolysis) of tissue slices, when the activity is based on the wet weight, dry weight or nitrogen content of the tissue. However, there is some question as to whether these observations reflect changes occurring during growth or those occurring during senescence. On the other hand, a lack of agreement appears to exist when the activities are based on cell count. These discrepancies may be due to species differences, tissue differences or methods of determining cell number.

Although some data suggest that senescence may be accompanied by a decreased oxygen

uptake of the individual cells, measurements of the activity of specific enzymes within the Krebs cycle lend no support to this postulate. However, it should be recalled that no experiment which demonstrated an agewise decreased oxygen uptake per cell attempted to measure any of these specific enzymes. Therefore, the key experiment essential to answering this question remains to be carried out.

Finally, the data, covering a number of points in the life span, suggest a general pattern of changes in the concentration of various enzymes. This may be characterized by an increasing concentration during the early part of life, a leveling off, then a decreasing concentration in the latter part of life. However, taken as a whole, these data fail to answer the fundamental question as to whether the decreased concentration, observed in the senescent animal, may be attributed to a loss of cells or cellular elements from the tissues; an increase in acellular elements, or whether there is a reduction in the metabolic activity of the individual cell with increasing age.

REFERENCES

1. Wollenberger, A. and J. Jehl. *Am. J. Physiol.* 170: 126, 1952.
2. Pearce, J. M. *Am. J. Physiol.* 114: 255, 1936.
3. Jacob, M., L. Mandel and P. Mandel. *Experientia* 10: 218, 1954.
4. Ross, M. H. and J. O. Ely. *J. Franklin Inst.* 258: 63, 1954.
5. Hawkins, J. *J. Gen. Physiol.* 11: 645, 1927.
6. Rosenthal, O., M. A. Bowie and G. Wagoner. *J. Cell. & Comp. Physiol.* 17: 221, 1941.
7. St. Aubin, P. M. G. and N. R. L. Bucher. *Anat. Rec.* 112: 797, 1952.
8. Rafsky, H. A., B. Newman and A. Horonick. *J. Gerontol.* 7: 38, 1952.
9. Reiner, J. M. *J. Gerontol.* 2: 315, 1947.
10. Lawrie, R. A. *Nature.* 170: 122, 1952.
11. Dyrbye, M. and J. E. Kirk. *J. Gerontol.* 11: 33, 1956.
12. Lightbody, H. D. *J. Biol. Chem.* 124: 169, 1938.
13. Zorzoli, A. *J. Gerontol.* 10: 156, 1955.
14. Clark, L. C., Jr, E. I. Beck and N. W. Shock. *J. Gerontol.* 6: 7, 1951.
15. Rockstein, M. *J. Cell. & Comp. Physiol.* 35: 11, 1950.
16. Rockstein, M. *Biol. Bull.* 105: 154, 1953.

COMPARATIVE PHYSIOLOGY OF AGING

A. I. LANSING[1]

From the Department of Anatomy, Emory University, Emory, Georgia

I HAVE generally defined aging as a process associated with the adult organism, usually correlated with the passage of time, resulting in unfavorable changes and terminating invariably with death of the organism (1). This point of view, and it is no more, makes several assumptions: that aging is an endogenous process, that aging begins in adulthood, that aging generally results in deleterious changes in the organism, and that death of the individual may result from aging in the absence of degenerative diseases. Small oases of data exist to substantiate these points but by and large one must regard the previous statement as no more than a working hypothesis.

From the point of view of the experimental biologist, it seems essential to determine whether endogenous factors operate to produce aging on whether external factors, such as diseases or accidents, operate to interfere with indefinite vigorous life of the individual. It is obvious that in the sense I am using the term 'aging' I am referring only to the process of change with time in the life of the adult organism, i.e. senescence. I am not including the developmental changes that occur with the passage of time in the life of the fertilized ovum through the period of adolescence. The question represents more than formal argument. Like the philosopher, the experimentalist cogitates and formulates hypotheses. Unlike the philosopher, the experimentalist uses the hypothesis to lead him into an experiment. An hypothesis which does not lead to experimentation does little for the investigator. Other things being equal, it would follow that the sounder the hypothesis, the more fruitful the experiment is apt to be.

It is probably true that in the interests of a species, aging, whether it be endogenous or exogenous, is an advantageous adaptation. Survival beyond the period of reproductive capability is a luxury a species can ill afford. While much rhetoric can evolve from such reasoning, the question still remains—why is the life span of most species finite? It appears to me that a number of observations made in several of the phyla indicate that aging is more than an accumulation of environmental insults.

In some protozoans, it seems clear that decline and death can be averted by nuclear reorganization resulting from either conjugation or autogamy. In the absence of both of the latter, decline in fission rate will result. If by experimental manipulation, the interval between successive autogamies is increased, there is a significant decrease in vigor as measured by fission rate.

Quite recently, Sonneborn (2) has analyzed the viability of fission products of paramecia formed immediately after and immediately before autogamy. The paramecia, obtained just after autogamy, may be regarded as young, while those produced just before autogamy are old. In spite of thoroughly standardized environmental conditions, Sonneborn found that the ability of paramecia to survive autogamy depended upon their age. The mortality of old paramecia in autogamy was high and was attributed to the production of chromosomal aberrations.

In other protozoa as an Ameba polypoda, the life of the individual extends from one fission to the next. Hartmann in 1922 (3) found that, by surgical amputation and subsequent regeneration of cytoplasm, fission and loss of individuality could be avoided. Thus, 32 amputations over a 42-day period prevented fission while another individual from the same lineage went through 15 fissions.

Hartmann was able to obtain similar results with the multicellular flatworm, Stenostomum leucops, which also reproduces by transverse fission. This organism was kept alive for 13 months by 52 repeated amputations of the anterior end of the animal during which time sister animals had undergone 41 divisions.

Using a related flatworm, Stenostomum incau-

[1] Present address: Department of Anatomy, University of Pittsburgh, Pittsburgh, Pennsylvania.

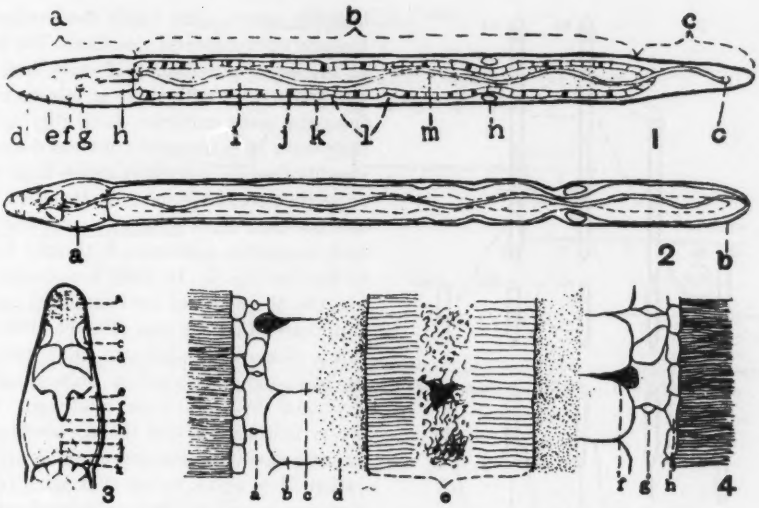


FIG. 1. 1: Diagram showing characters commonly found in the genus Stenostomum. *a*, Head; *b*, trunk; *c*, tail; *d*, ciliated pit; *e*, cerebral ganglion; *f*, light-refractive organ; *g*, mouth; *h*, pharynx; *i*, intestine; *j*, intestinal gland; *k*, pseudocoel; *l*, constrictions of intestine marking places where new heads are just beginning to form; *m*, protonephridium; *n*, anlage of cerebral ganglion of new zooid; *o*, nephridiopore. 2: Diagram of S. incaudatum showing specific differentiae (magnification, seventy diameters). *a*, Pharynx, short, wide, and lacking glands; *b*, posterior part of trunk, lacking tail. 3: Head of S. incaudatum (magnification, one hundred seventy-five diameters). *a*, Transverse muscles; *b*, ciliated pit; *c*, ciliated-pit ganglion; *d*, cerebral ganglion; *e*, mouth; *f*, pharynx; *g*, neck of flask-shaped lumen of pharynx; *h*, lumen of pharynx; *i*, sphincter in pyramidal mass extending from posterior wall of pharynx; *j*, base of flask-shaped lumen of pharynx; *k*, posterior wall of pharynx. 4: Diagram of longitudinal section through part of trunk (magnification, one thousand diameters). *a*, Pseudocoel; *b*, globule; *c*, distal part of intestinal cell; *d*, proximal part of intestinal cell full of food in process of digestion; *e*, ciliated lumen of intestine, containing food and feces; *f*, intestinal gland; *g*, transverse muscle; *h*, ciliated epidermis. (From T. M. Sonneborn. *J. Exper. Zool.* 57: no. 1.)

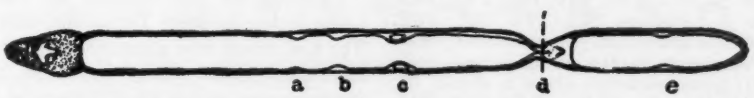


FIG. 2. Fission characteristics of S. incaudatum. Chain of zooids about to divide at plane indicated by broken line (*d*). Magnification, 70 diameters. *a*, *b*, *c*, *d*, *e*, Constriction of the intestine marking regions in which heads of zooids are developing. (From Sonneborn, *loc. cit.*)

datum, Sonneborn (4) did an ingenious selection experiment. In its transverse fission, Stenostomum produces anterior and posterior daughters; the two are not alike (figs. 1 and 2). The anterior product has only to regenerate a small tail component while the posterior daughter has to grow a new trunk and head portion. In successive anterior products, obtained by repeated selection from isolation culture, there are then the same head and new tails while in their posterior counterparts there are new heads and trunks but the same tail (fig. 3). In one, there is a limited amount of growth activity; in the other, there is an abundance of growth. Anterior products show many structural abnormalities and the anterior lines died out in 35 days, while the posterior lines indicated indefinite life spans (table 1). These differences in vitality existed in spite of carefully standardized environmental conditions.

Several years ago a related observation was made by me using the rotifer as the experimental organism (5). Because this animal is parthenogenetic, it was simple to obtain a genetically homozygous stock. The animals were raised in

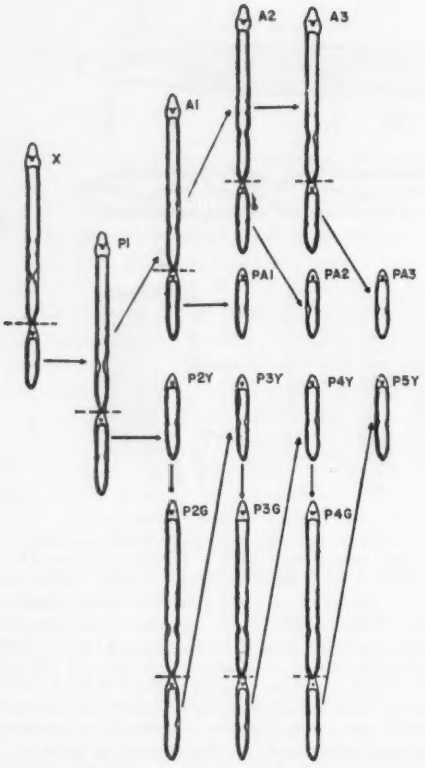


FIG. 3. Method of selecting lines of anteriors and lines of posteriors, showing difference in size between posteriors produced by anteriors and posteriors produced by posteriors. X: Ancestor of both lines. P1, P2Y, P2G, P3Y, P3G, P4Y, P4G, P5Y: line of posteriors. P2Y, P3Y, P4Y, P5Y: young posterior individuals just produced by division of individuals in the posterior line. P2G, P3G, P4G: grown posteriors about to divide. P1, A1, A2, A3: line of anteriors. PA1, PA2, PA3: young posterior individuals just produced by division of individuals in the anterior line. Broken lines indicate planes of division.

TABLE 1. COMPARISON OF MEAN LENGTH OF LIFE OF LINES OF ANTERIORS WITH MEAN LENGTH OF LIFE OF LINES OF POSTERIORS

	Lines of Anteriors			Lines of Posteriors	
	No. of lines	Mean length of life, days		No. of lines	Mean length of life, days
All	388	35.0±1.1	All	8	79.8+
			Those which died	4	63.5
Group 5A (group with highest mean)	47	68.9±2.0	Those which were lost	2	77.0
			Those which were discarded	2	115.0+

isolation culture using highly standardized nutritive and environmental conditions. The life spans of successive generations of early-born rotifers were contrasted with those of late-born rotifers from the same maternal stock (fig. 4). In the early-born lines, longevity increased slowly but steadily through successive generations to quadruple the normal life span. On the other hand, in the late-born lines, longevity was shortened in each successive generation with final extinction of the line (fig. 5). In these experiments, in the absence of genetic or environmental variations, it appeared that the eggs of adult rotifers transmit a capacity to accelerate aging which is cumulative and also reversible. This capacity is not expressed by eggs from adolescent mothers. Every indication is that the age-accelerating capacity makes its appearance abruptly upon maturation. Here again, we see that aging potentialities may be and can be manipulated even under rigidly regulated environmental conditions.

In 1933, Ingle (6) induced partial starvation in Cladocerans by the simple expedient of diluting the infusion medium with pond water. Although marked increases in longevity were observed in the semi-starved animals, Ingle attributed the extension of total life span to a delayed maturation. Kopec (7) investigated the effect of partial starvation upon caterpillars of Lymantria, Drosophila and tadpoles and found that extension of total duration of life is effected, not by an increase in the period of adult life, but by an extension of the period of growth.

Northrop (8) demonstrated an endogenous aging factor in other Drosophila experiments. The flies, which were genetically regulated, were maintained under aseptic conditions but temperatures were varied. At 10°C the total duration of life was 177.5+ days, at 20°C total duration was 54.3 days and at 30°C total duration of life was 21.15 days (table 2). Apparently the rate of living determines longevity, at least in fruit flies.

There are few experiments on aging that have been done on phyla between the insecta and mammals. The rat has been used in a variety of experiments which are largely nutritional. The work of Strong (9, 10) parallels to a significant degree the studies on rotifers. Whereas maternal age in rotifers influences the longevity of offspring, Strong has found that in inbred strains of mice maternal age significantly affects tumor incidence, rate of metastasis, rate of

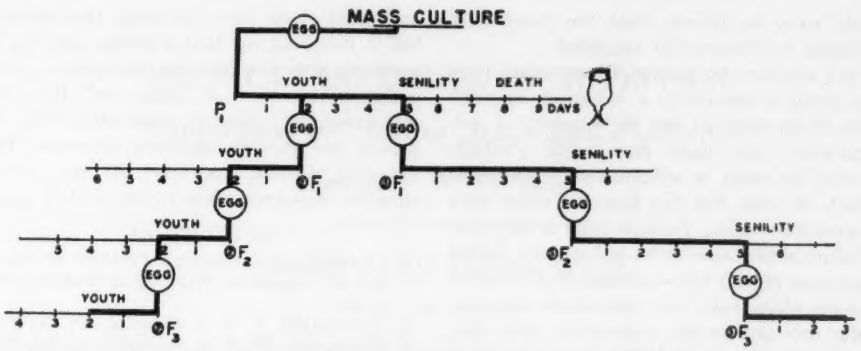


FIG. 4. Orthoclone method, showing the procedure developed for selecting successive generations of a parthenogenetic rotifer with constant parental age in each generation (Euchlanis triquetra).

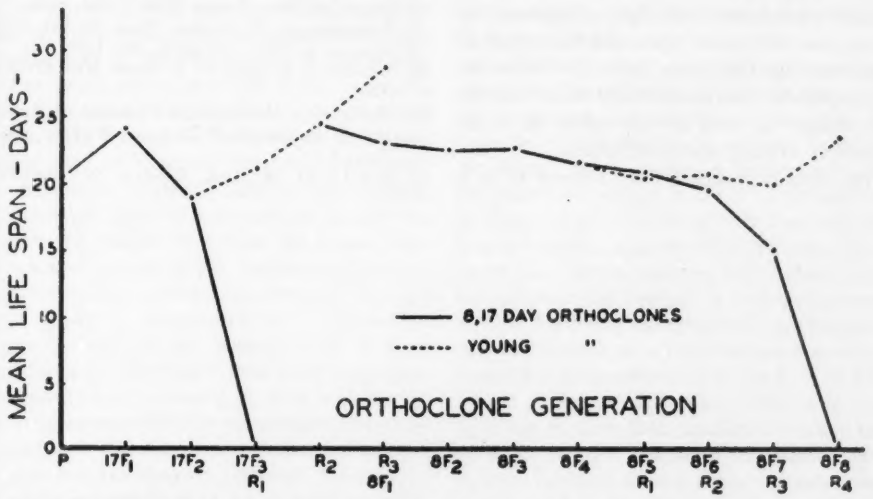


FIG. 5. Series 2 of Philodina citrina showing accelerated aging in old orthoclones and reversal by establishment of offshoot young orthoclones.

TABLE 2. EFFECT OF TEMPERATURE ON DURATION OF LIFE OF DROSOPHILA (AFTER NORTHROP)

Temperature °C	Duration (in days) of Life of Imago	Total Duration of Life
10	120.5	177.5 + x
15	92.4	123.9
20	40.2	54.3
25	28.5	38.5
27.5		
39	13.6	21.15

TABLE 3. CONCENTRATION OF PHOSPHORUS IN VARIOUS FRACTIONS OF MOUSE LIVER (ADAPTED FROM SCHULZ)

Age Group	Acid-Soluble P	Ribo-P	Lipide P	Dexoxy-P	Total P
0–2 months	73.4	109.8	107.3	24.4	360.9
3–6 months	71.4	105.3	109.5	22.8	354.1
10–13 months	79.2	101.3	111.4	23.9	368.3

tumor growth, etc. McCay's experiments (11) on rats are now well known to most of us. Rats were raised on a diet containing all of the essential nutritional material but lacking sufficient calories to promote growth and maturation. These rats were maintained on the growth-limiting diet for 766 and 911 days after which time an increase of caloric intake was able to initiate rapid growth. These previously retarded rats lived as much as 200 days longer than their litter-mate controls. Once again, the evidence

would seem to indicate that the potentiality for aging is endogenously regulated.

On a number of occasions, I have pointed out that aging is essentially a biological anomaly. Since living material has the capacity of self-duplication, one might expect this synthetic capacity to result in self-renewal for unlimited periods of time. Yet this does not occur since we experience aging. Perhaps aging is the result of failure of the capacity for self-renewal. In this regard, one quickly turns attention to the nucleoproteins which have been associated with synthetic processes in the expectation that they might show age changes. This does not appear to be the case. In a recent study, Schulz (12) has analyzed the amounts of RNA, DNA, acid-soluble phosphorus and lipid phosphorus in young and old mouse livers and has found no significant age differences (table 3). Either the nucleoproteins then are not involved, or else the age changes in them are too subtle to be detected by existing analytical means.

The several studies I have referred to in a variety of phyla have obviously been selected but it would appear that a strong case can be made for the view that an endogenous mechanism is operative in aging and that this mechanism is intimately associated with the growth and growth regulatory processes. This reasoning, if valid, may pave the way to constructive experimentation in the field of aging.

REFERENCES

1. Lansing, A. I. *Cowdry's Problems of Aging*, 3rd ed. Baltimore: Williams & Wilkins, 1952, chap. 1.
2. Sonneborn, T. M. *J. Gerontol.* 10: 470, 1955.
3. Hartmann, M. *Biol. Zentralbl.* 42: 364, 1922.
4. Sonneborn, T. M. *J. exp. Zool.* 57: 57, 1930.
5. Lansing, A. I. *J. Gerontol.* 2: 228, 1947.
6. Ingle, L. *Science* 78: 511, 1933.
7. Kopec, S. *Brit. J. exp. Biol.* 5: 204, 1928.
8. Northrop, J. H. *J. Biol. Chem.* 32: 123, 1917.
9. Strong, L. C. *J. Gerontol.* 340, 1951.
10. Strong, L. C. *Ann. N. Y. Acad. Med.* 57: 507, 1954.
11. McCay, C. M. *Cowdry's Problems of Aging*, 3rd ed. Baltimore: Williams & Wilkins, 1952, chap. 6.
12. Schulz, D. M. *Arch. Biochem.* 27: 57, 1950.

AMERICAN INSTITUTE OF NUTRITION

Symposium on Amino Acids and Proteins[1]

Chairman: W. C. ROSE

AMINO ACID IMBALANCE

C. A. ELVEHJEM

From the Department of Biochemistry, University of Wisconsin, Madison, Wisconsin

AMINO ACID IMBALANCE in animal nutrition has not received extensive consideration (1). On the basis of past experiences with the quantitative relationships between other nutrients, and the scattered data reported so far on amino acids, there is every reason to expect an adverse effect from an improper balance between two or more amino acids. As early as 1928 Jackson, Sommer and Rose (2) attempted to improve the quality of gelatin by adding the amino acids which were missing or low. Because of the negative results the authors suggested that the data were probably complicated by a deleterious action exerted by the relatively high level of gelatin used in the diet. Within a few years after this observation, especially in work with chicks, it was suggested that the requirement of certain amino acids was increased as the level of protein in the diet was elevated. However, the expense of using combinations of amino acids in order to study these relationships further has delayed the work with animals. Since the cost of amino acids is much less when microorganisms are used, a number of imbalances have already been demonstrated with bacteria (1).

The results which I can present here are preliminary in many respects but further work will surely give us true explanations for these relationships. Such knowledge will not only be of value in normal nutrition, but may give us methods of controlling the growth of viruses and tumors in animals without significantly affecting the host. As our knowledge increases, there will undoubtedly be many individual relationships, but I would like to discuss these relationships in this paper under the following three conditions.

1) The increase of a given amino acid may precipitate the deficiency of the next most limiting amino acid. The symptom of the deficiency may be evident through a decrease in growth or through other metabolic disturbances. The production of beriberi in a rat by the addition of riboflavin to a diet low in both these vitamins is a well-known fact. Since the deficiency of an amino acid seldom produces such distinct external symptoms as beriberi, it may be necessary to look for tissue changes, such as changes in enzyme systems or accumulation of metabolic products such as neutral fat in the liver, in addition to growth changes. Much evidence is accumulating to show that significant changes may occur in different tissues of an animal before there is evidence of any change in total body weight when amino acids are not supplied at the proper levels.

2) One amino acid may be antagonistic to another amino acid, and as the intake of the first amino acid is increased the requirement for the other amino acid shows a corresponding increase. In this case an increase of the second amino acid should completely overcome the effect produced by the increase of the first amino acid.

3) A specific toxic effect may be produced. In this case another amino acid may not have a specific or direct effect in overcoming this toxicity. A supplement of an amino acid or other nutrients, such as vitamins, may have some indirect effect on the rate of detoxification.

In each condition the effect may be a metabolic

[1] Atlantic City, April 18, 1956.

one or the effect may be upon the degree of absorption of the original amino acid from the intestinal tract. The latter effect is more likely to occur in condition number one than in the other two conditions. Furthermore, if we use a protein as a source of the amino acids studied, we may also encounter the problem of availability of the amino acid from the protein. In this paper no attempt will be made to follow a chronological order of the development of our knowledge of these interrelationships, but I shall try to give specific examples of each of the conditions which I have described.

It is now clearly established that the level of threonine in the diet significantly affects the tryptophan requirement. When we use a diet complete in all other respects but containing 9% of casein supplemented with 0.2% of cystine or methionine as a sole source of protein, the growth rate is about 12 gm/wk. I should perhaps make one modification in the statement that the diet is complete in all other respects since in studies with tryptophan the niacin must also be controlled; therefore, in the diets described here the niacin is omitted. If 0.078% of DL-threonine is added to this diet, the growth is reduced to 4 gm/wk. The addition of 0.2% DL-tryptophan increases the growth to 19–20 gm/wk. These figures are taken from data obtained in our own laboratory (3). However, Singal and coworkers (4) obtained exactly similar data using 0.4% DL-methionine and 0.2% L-tryptophan. When tryptophan was omitted from this diet the growth was 3 gm/day. When the tryptophan was included, the growth increased to 23 gm/day. Henderson and coworkers (5) at Illinois have obtained similar results using a diet containing 10% hydrolyzed casein supplemented with 0.1% histidine and 0.1% DL-tryptophan. Upon addition of 0.1% DL-threonine, the growth was reduced significantly, and upon addition of 0.12% of L-tryptophan the rate of growth was increased fourfold.

The actual relationships between these amino acids are given in table 1. It is apparent that 18% of casein supplies the accepted requirements for threonine, cystine and methionine and tryptophan. When the level of casein is reduced to 9%, each of these amino acids is supplied at a level below the requirement and the growth is reduced from 32 to 10 gm/wk. The addition of 0.2% cystine increases the intake of this amino acid to a level approaching the requirement but by doing so the growth is increased only slightly. If 0.04% threonine is added to this ration, the threonine and the tryptophan intakes are still suboptimum, but this small increase in the level of threonine reduced the growth to 4 gm/wk. Henderson *et al.* (5) commented similarly that the threonine level which causes marked growth depression with either a casein or acid hydrolyzed casein diet is approximately 0.4%, 0.34% from the casein and 0.05% from the L-isomer corresponding to 0.1% DL-threonine. With the addition of 0.1% tryptophan, which increases the total intake of this amino acid above the accepted requirement, the growth is increased to 19 gm/wk.

TABLE 1. LEVELS OF AMINO ACIDS ON GROWTH

Casein	Additions			Levels in Diet			Gain/ week
	Threonine	Cystine and methionine	Tryptophan	Threonine	Cystine and methionine	Tryptophan	
%	%	%	%	%	%	%	*gm*
R				0.50	0.60	0.20	
18				0.68	0.54	0.22	32
9				0.34	0.27	0.11	10
9		0.2		0.34	0.47	0.11	12
9	0.04	0.2		0.38	0.47	0.11	4
9	0.04	0.2	0.1	0.38	0.47	0.21	19
9	0.20	0.2		0.54	0.47	0.11	3
9	0.20	0.2	0.1	0.54	0.47	0.21	23
9	0.2			0.54	0.27	0.11	12

R = Requirement.

These data demonstrate a most sensitive balance between threonine and tryptophan and the amount which causes a significant effect on growth is very small indeed. If the added threonine is increased to 0.2%, which then raises the intake to approximately the required amount, the degree of inhibition is no greater, about 3 gm/wk. Again the addition of tryptophan completely counteracts even this high level of threonine. If 0.2% threonine is added without the cystine supplement and without tryptophan, the growth inhibition is not nearly as great and 12 grams growth per week is obtained. Thus the very sensitive response to slight increases of threonine depends upon the presence of cystine or methionine in the diet at fairly normal levels. Again I should point out that in all these calculations tryptophan has been used to counteract the effect of threonine. Nicotinic acid works equally as well, because if the diet contains 0.1% tryptophan the additional requirement of tryptophan can be supplied as nicotinic acid.

Approximately 1 mg of the nicotinic acid is equivalent to 50 mg of tryptophan under these conditions. We have, then, an example where the level of one amino acid may seriously affect the requirement for the next most limiting amino acid. In this case we have used growth as the criteria for measuring the effect.

Let us now turn to another example of this relationship, but in this case we will use a metabolic disturbance rather than growth as the criterion If we use the same 9% casein diet and supplement it with niacin, the growth rate will be about 15 gm/wk., but after a period of 2–3 weeks the level of fat in the livers of these animals will increase to 25–30% on a dry weight basis (6–8). The level of fat in the livers of weanling rats is 8–10% and in normal rats after 2 weeks on an adequate diet would be 10–14%. If the level of protein is increased by adding 6% gelatin, the liver fat returns to normal although there is only slight growth response. If 2% of casein is added, again the liver fat approaches normal but there is a more significant growth response. It appears then that certain amino acids may have a more significant effect on growth than on accumulation of fat in the liver. It has now been clearly demonstrated that the level of liver fat can be reduced to normal by the addition of 0.18% of DL-threonine. A level of 1.5% of glycine produces a considerable effect but generally does not reduce the level of liver fat to normal Again the sulfur amino acids enter into this relationship since in the absence of cystine and methionine supplements liver fat is only slightly elevated, but upon addition of 0.1–0.3% DL-methionine the liver fat is increased to 26% (9). The increase is completely counteracted by the addition of threonine.

These relationships are shown in table 2. It is apparent from the data in this table that 9% casein plus 0.2% cystine or methionine produces a liver fat of about 30% after a period of 2 weeks. In this case the threonine and tryptophan are inadequate to meet the requirements, although with the added niacin the tryptophan requirement should be adequate. It also shows that the addition of 0.05% tryptophan to such a diet has little or no effect on the liver fat. However, the addition of 0.09% threonine, which increases the intake to approximately the required level, produces a normal liver fat. By omitting the methionine from the casein diet, the level of liver fat is also reduced, but the addition of only 0.05% of methionine, which gives a level of intake which is still below the required amount, increases the liver fat to about 25%. A still higher level of methionine will not increase the liver fat if threonine is supplied in the diet. It appears then that a low level of threonine in the diet not only reduces growth of the animal but may have a significant effect on the liver fat. The accumulation of fat appears only when there is an adequate amount of methionine in the diet.

TABLE 2. LEVELS OF AMINO ACIDS ON LIVER FAT

Casein	Additions			Levels in Diet			Liver fat % dry weight
	Threonine	Cystine and methionine	Tryptophan	Threonine	Cystine and methionine	Tryptophan	
%	%	%	%	%	%	%	
R				0.50	0.60	0.20	
18				0.68	0.54	0.22	12.0
9		0.2		0.34	0.47	0.11	30.1
9		0.2	0.05	0.34	0.47	0.16	28.0
9	0.09	0.2		0.43	0.47	0.11	12.4
9				0.34	0.27	0.11	16.4
9		0.05		0.34	0.32	0.11	24.6
9	0.18	0.30		0.52	0.57	0.11	14.0
							Fresh wt.
Amino acid mix*				0.27	0.55	0.31	12.8
Amino acid mix	0.20			0.47	0.55	0.31	5.6

R = Requirement.
* Singal *et al.*, *J. Biol. Chem.* 200: 883, 1953.

These relationships may not be quite as simple as I have indicated since the use of proteins other than casein in the basal diet does not give such clear cut results. For example, when blood fibrin is used as the protein there is no accumulation of liver fat even when the level of fibrin is reduced to 6% (10). This may be explained on the basis that fibrin contains appreciably more threonine than casein. This could easily be answered by supplementing 6% of fibrin with other amino acids in order to increase the rate of growth and then determine if fatty livers would be produced with the same level of threonine as that provided by casein. Similarly with 9% of egg albumin there is a definite increase in liver fat. The addition of 0.18% of L-threonine does not reduce the liver fat significantly. On the other hand, 1.5% of glycine does have a definite effect. This may be due to the balance of other amino acids or it may possibly be due to the relatively high level of sulfur amino acids in egg albumin. It may be important to mention that the addition of lysine and histidine together with threonine to albumin

TABLE 3. AMINO ACIDS SUPPLIED BY CEREALS

	Protein in Diet	Threonine	Cystine	Methionine	Total Cystine and Methionine	Lysine	Tryptophan	Histidine	Growth, Gain/Wk	Liver fat
	%	%	%	%	%	%	%	%	gm.	%
90% rice	5.9	.226	.07	.133	.203	.221	.06	.094	8.8	32
90% corn	8.4	.335	.107	.156	.263	.239	.05	.171	2.2	12
88% wheat	9.1	.243	.185	.13	.315	.232	.102	.172	9.9	12
9% casein	7.8	.34	.031	.237	.268	.630	.106	.230	10	16

diets reduces the liver fat to normal (10). Lysine is not active unless the other amino acids are present.

Interesting differences have also been obtained with cereal proteins (11). When rice is used as a sole source of protein in otherwise complete diets, there is appreciable accumulation of liver fat. However, when either corn or wheat is used the level of liver fat does not increase above the normal range. The data in table 3 show that the rice diet supplies only slightly less threonine than the wheat diet and therefore the difference cannot be explained entirely on the difference in the threonine intake. Apparently the levels of cystine and methionine are not responsible since rice supplies less cystine and methionine than do the other two cereal proteins. It is possible that the level of histidine may be important since rice supplies less histidine than either corn or wheat. The implication of histidine seems more probable when we find that 9% of casein supplies 0.23% of histidine and 9% of egg albumin only 0.16% of histidine. I mentioned earlier that histidine seemed to have a beneficial effect on reducing liver fat in egg white diets. We should emphasize again that in all these cases we are dealing with an intake of each amino acid below the accepted requirement; for example, the histidine requirement is 0.4%. It is important that *condition 1* can be produced using a variety of different proteins and that several combinations of amino acids may be involved.

Let us now turn to the second condition of amino acid imbalance, namely, a true antagonism between two amino acids. When 3% L-leucine is added to a 9% casein diet, there is a very significant decrease in growth rate (12). When a similar level of leucine is added to a diet containing 18% of casein, a growth retardation is not observed. It is obvious, therefore, that the extra level of casein supplies additional amounts of an amino acid which counteracts the increased levels of leucine. Further work showed that the active amino acid is isoleucine. The addition of 0.4% DL isoleucine will counteract approximately 1.5% of L-leucine. However, with the higher level of L-leucine, 3%, the increase of isoleucine from 0.4 to 0.8% does not give complete counteraction. Further work has shown that valine will completely overcome the growth inhibition.

Some of these relationships are shown in table 4. It is obvious that 9% casein supplies sufficient leucine and isoleucine to meet the requirements, and therefore we are dealing with a true antagonism rather than a limiting amount of the second amino acid. Some question might be raised about valine since the intake of this amino acid is borderline. However, since leucine, isoleucine and valine have very similar structures it is not surprising that there is a true antagonism between them. It is true that higher levels of leucine are needed to affect the valine requirement than are needed to affect the isoleucine requirement. Further data (13) show that valine, isoleucine and phenylalanine reduce the utilization of leucine for the growth of rats fed a 9% casein diet supplemented with a number of other amino acids.

TABLE 4. LEVELS OF AMINO ACIDS ON GROWTH

Casein	Additions			Levels in Diet			Gain/wk.
	Leucine	Isoleucine	Valine	Leucine	Isoleucine	Valine	
%	%	%	%	%	%	%	gm
R				0.8	0.5	0.7	
18				1.61	1.03	1.13	32
9				.80	.51	.56	18
9	3.0			3.80	.51	.56	4
9	3.0	0.4		3.80	0.91	.56	13
18	3.0			4.61	1.03	1.13	30
9	1.5			2.30	.51	.56	9
9	1.5	0.2		2.30	.71	.56	18
9	3.0	0.6		3.80	1.11	.56	8
9	3.0	0.6	0.6	3.80	1.11	1.16	15

R = Requirement.

These relationships appear to be identical to those reported recently for bacteria by Dien *et al.* (14).

One may immediately conclude from the fact that similar data are obtained with rats and bacteria that the antagonism is a metabolic one. This may or may not be true. It is still important to determine whether one amino acid, that is, leucine, may affect absorption of isoleucine from the intestinal tract of the animal or from the medium into the bacterial cell. In this connection it might be interesting to point out that studies of these relationships may be complicated by the fact that one of the amino acids in the pair may be less available in natural foodstuffs than the other. This is particularly true in studies on leucine-isoleucine relationships if zein from corn is used as the protein. According to analytical data the ratio of leucine to isoleucine in zein is within a range that should not produce antagonism. However, a very definite growth response is obtained when extra amounts of isoleucine are added to corn or to zein if other lacking amino acids are added. Further work has shown that the isoleucine in zein is relatively unavailable (15). Studies with the rat have shown that less than 25% of the total isoleucine present in zein can be utilized by the rat.

Further antagonisms have been observed between phenylalanine, tyrosine and threonine (16). The growth of rats fed a 9% casein diet supplemented with tryptophan and methionine is markedly depressed by adding either 3% DL-phenylalanine or 3% of tyrosine. A supplement of threonine prevented this growth depression whether phenylalanine or tyrosine was used. Threonine was only partially effective against a more severe growth depression produced with 5% of DL-phenylalanine. Furthermore threonine did not prevent the growth depression caused by phenylalanine when tryptophan was not added to the diet. These relationships are not clearly established since one would assume that if phenylalanine increased the requirement for threonine one would observe one of the symptoms of threonine deficiency, namely fatty livers. Actually the addition of phenylalanine to the basal diets reduced the level of liver fat rather than increased the level. Thus the relationship may be different from that of merely increasing the requirement for threonine.

Finally we come to *condition 3*, namely, a specific toxic effect may be produced. In this connection one must refer to a recent paper from Dr. Greenstein's laboratory (17) in which the toxicity of individual essential L-amino acids was determined. The amino acids were administered intraperitoneally into adult male rats which had been starved for 24 hours prior to the treatment. The LD_{50} levels for 12 different amino acids were determined and it was found that isoleucine was most toxic and tryptophan was least toxic. The LD_{50} for L-leucine was found to be 41 mM/kg. It is interesting that the addition of 3% of L-leucine plus that present in the diet brings the L-leucine intake to 54 mM/kg/day. Thus the intake is even higher than that used by Greenstein but it is ingested orally and spread over a 24-hour period. In a later paper from Greenstein's laboratory (18) it is pointed out that L-arginine strikingly reduced the toxicity of a mixture of other amino acids and that the role of L-arginine may be related to its protection against ammonia toxicity. This may suggest that at these high levels of amino acid injection, and I should emphasize that all of this work was done upon injection of amino acids, the toxicity may be quite different than that observed when the amino acid is ingested orally over longer periods of time.

Time will permit only the mention of two amino acids in this category. As early as 1932 Sullivan, Hess and Sebrell (19) reported growth inhibition and toxicity of glycine on a diet containing 4% of casein when the amino acid was fed from 2½ to 20% of the diet. Since the basal diet contained such a low level of protein, it would of course be possible that an imbalance might occur under these conditions. Hier, Graham and Klein (20) used both gelatin and casein diets and found significant growth inhibitions with 6% glycine, 5% of DL-phenylalanine, or 5% L-proline. These authors speak specifically of amino acid imbalance and since we may obtain inhibition with 5% of phenylalanine it would be interesting to determine if threonine would counteract the effect similar to that that I have already mentioned. However, more recent work with glycine seems to suggest that glycine is toxic especially since higher levels of folic acid seem to counteract this toxicity. Again we must be very careful in calling this a specific toxicity since an increased intake of folic acid may favor the utilization of glycine and thereby alter the level that can produce an imbalance. It appears more likely, however, that we are dealing here

with a toxic effect. The other amino acid which I would like to mention is methionine. The addition of 0.4% of methionine to an 18% casein diet causes a growth depression when vitamin B_6 is not supplied (21). Pyridoxine will counteract the effect of moderate amounts of methionine but at levels of 2.5% of methionine poor growth results even in the presence of large amounts of the vitamin. The higher levels of pyridoxine may facilitate the detoxification of methionine and its metabolites up to a given limit. Again, it is possible that this detoxification or alteration may reduce the level of methionine in the metabolic pool to a point where it would no longer have an effect on certain related amino acids.

In conclusion I would like to emphasize that data on amino acid imbalance has been accumulating slowly in the literature over the past 30 years. In the earlier work, the term 'imbalance' was not used but many of the results reported at that time can be definitely verified today under more carefully controlled conditions. With more accurate techniques we should be able to establish clearly which effects are due to toxicity, which are due to antagonisms between amino acids and which are more closely related to nutritional requirements.

REFERENCES

1. Harper, A. E., D. A. Benton and C. A. Elvehjem. *Arch. Biochem. and Biophys.* 57: 1, 1955.
2. Jackson, R. W., B. E. Sommer and W. C. Rose. *J. Biol. Chem.* 80: 167, 1928.
3. Hankes, L. V., L. M. Henderson, W. L. Brickson and C. A. Elvehjem. *J. Biol. Chem.* 174: 873, 1948.
4. Singal, S. A., V. P. Sydenstricker and J. M. Littlejohn. *J. Biol. Chem.* 176: 1063, 1948.
5. Henderson, L. M., O. J. Koeppe and H. H. Zimmerman. *J. Biol. Chem.* 201: 697, 1953.
6. Singal, S. A., S. J. Hazan, V. P. Sydenstricker and J. M. Littlejohn. *J. Biol. Chem.* 200: 867, 1953.
7. Harper, A. E., W. J. Monson, D. A. Benton, and C. A. Elvehjem. *J. Nutrition* 50: 383, 1953.
8. Harper, A. E., W. J. Monson, D. A. Benton, M. E. Winje and C. A. Elvehjem. *J. Biol. Chem.* 206: 151, 1952.
9. Harper, A. E., D. A. Benton, M. E. Winje and C. A. Elvehjem. *J. Biol. Chem.* 209: 159, 1954.
10. Winje, M. E., A. E. Harper, D. A. Benton, R. E. Boldt and C. A. Elvehjem. *J. Nutrition* 54: 155, 1954.
11. Harper, A. E., M. E. Winje, D. A. Benton and C. A. Elvehjem. *J. Nutrition* 56: 187, 1955.
12. Harper, A. E., D. A. Benton and C. A. Elvehjem. *Arch. Biochem. and Biophys.* 57: 1, 1955.
13. Benton, D. A., A. E. Harper, H. E. Spivey and C. A. Elvehjem. *Arch. Biochem. and Biophys.* 60: 147, 1956.
14. Dien, L. T. H., J. M. Ravel and W. Shive. *Arch. Biochem. and Biophys.* 49: 283, 1954.
15. Benton, D. A., A. E. Harper and C. A. Elvehjem. *Arch. Biochem. and Biophys.* 57: 13, 1955.
16. Benton, D. A., A. E. Harper, H. E. Spivey and C. A. Elvehjem. *Arch. Biochem. and Biophys.* 60: 156, 1956.
17. Gullino, P., M. Winitz, S. W. Birnbaum, J. Corfield, M. C. Otey and J. P. Greenstein. *Arch. Biochem. and Biophys.* 58: 255, 1955.
18. Greenstein, J. P., M. Winitz, P. Gullino and S. M. Birnbaum. *Arch. Biochem. and Biophys.* 59: 302, 1955.
19. Sullivan, M. X., W. C. Hess and W. H. Sebrell. *Pub. Health Rep.* 47: 75, 1932.
20. Hier, S. W., C. E. Graham and D. Klein. *Proc. Soc. Exper. Biol. & Med.* 56: 187, 1944.
21. DeBey, H. J., E. E. Snell and C. A. Baumann. *J. Nutrition* 46: 203, 1952.

METABOLISM OF GLYCINE: THE SUCCINATE-GLYCINE CYCLE

DAVID SHEMIN

From the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York City

MANY OVER-ALL ASPECTS of glycine metabolism are now known, and especially the metabolic pattern of the α-carbon atom of glycine in its utilization for the synthesis of other compounds has been established. The α-carbon atom of glycine, no longer attached to the carboxyl group, is utilized for the synthesis of porphyrins (1–4), the ureido groups of purines (5), the β-carbon atom of serine (6, 7) and for methyl groups (8, 9). This metabolic pattern is similar to that of the so-called 'C_1' compounds, with the exception that the latter cannot substitute for glycine in porphyrin synthesis. It would seem therefore that these apparently unrelated compounds (porphyrins, purines, serine, and methyl groups) have one common feature, namely, the participation of the α-carbon atom of glycine for their synthesis. The problem of glycine metabolism may then be confined, in this discussion, to the mechanisms by which its α-carbon atom is utilized for the different compounds mentioned and to the mechanisms by which its α-carbon atom is detached from its carboxyl group. If we assume, for the purpose of discussion, that in the pathway of glycine metabolism intermediates are produced which are utilized both for porphyrin synthesis and for the synthesis of the other compounds, then postulated mechanisms of glycine metabolism which do not produce intermediates which are utilized for porphyrin synthesis must be looked upon with a certain amount of caution.

A pathway for glycine metabolism suggested itself from a study of the mechanism of porphyrin synthesis. In this pathway, called the succinate-glycine cycle (fig. 1; refs. 10, 11), it is postulated that 'active' succinate condenses on the α-carbon atom of glycine to give rise to α-amino-β-ketoadipic acid. The β-keto acid decarboxylates to give rise to δ-aminolevulinic acid, which can be utilized for porphyrin synthesis or be further catabolized in such a manner that its δ-carbon atom (originally the α-carbon atom of glycine) is utilized for the synthesis of the ureido groups of purines, the β-carbon atom of serine, and for methyl groups, while the remaining four-carbon-atom residue is reconverted to succinate. Since this succinate-glycine cycle was arrived at from our study of porphyrin synthesis, it may then be worthwhile to consider the pertinent facts of prophyrin synthesis which led to the elaboration of this cycle, and the findings which lend their support and broaden the implications of the postulated series of reactions.

It has been previously demonstrated that although the four nitrogen atoms of protoporphyrin are derived from glycine (12–16), eight carbon atoms of the porphyrin molecule are derived from the α-carbon atom of glycine (2–3); four of these carbon atoms are still attached to the nitrogen atom (3) and four are not (3, 4). The carbon atoms of protoporphyrin derived from the α-carbon atom of glycine are shown in figure 2. Furthermore, it has been found that the carboxyl group of glycine is not utilized for any of the carbon atoms of the porphyrin (2, 17). It has also been demonstrated that a succinyl intermediate, arising from the tricarboxylic acid cycle, is the source of the remaining 26 carbon atoms of protoporphyrin (18, 19). The relationship of the tricarboxylic acid cycle to porphyrin formation was formulated previously as shown in figure 3. This relationship was further documented by studying the synthesis of protoporphyrin from α-ketoglutarate-5-C^{14}, α-ketoglutarate-1,2-C^{14} and citrate-1,5-C^{14}. In each case the predicted carbon atoms of the porphyrin were labeled from the C^{14}-labeled substrate (20). The experiments in these studies were carried out by incubating either whole (21, 22) or hemolyzed red blood cells (18, 23, 24) of a duck with the C^{14}-labeled compounds and subsequently chemically degrading the isolated hemin. The degradation was done in such a manner that individual carbon atoms from a particular position in the porphyrin could be isolated (3, 19). The carbon atoms arising from succinate and glycine are

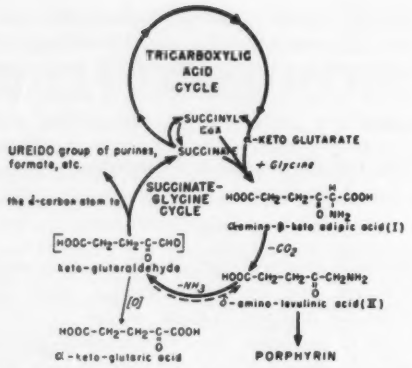


FIG. 1. The succinate-glycine cycle: a pathway for glycine metabolism.

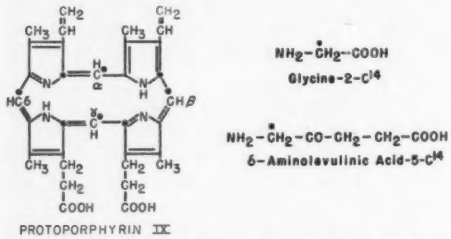


FIG. 2. Positions in protoporphyrin derived from the alpha-carbon atom of glycine.

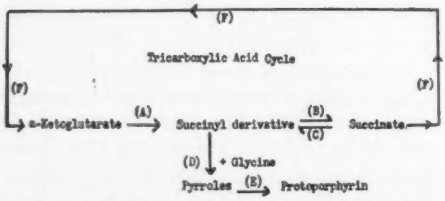


FIG. 3. Relationship of the citric acid cycle to protoporphyrin formation.

shown in figure 4. It would appear merely from the C^{14}-labeling pattern obtained that each pyrrole unit is made up of two moles of succinate and one mole of glycine (18). Since the methene bridges also arise from the α-carbon atom of glycine, the synthesis of the porphyrin molecule requires eight moles of both succinate and glycine.

It then became of interest to find the chemical mechanism by which the succinate and glycine combine to form the pyrrole unit of the porphyrin. Our studies on the utilization of the α-carbon of glycine for porphyrin formation have led us to the following mechanism. It was

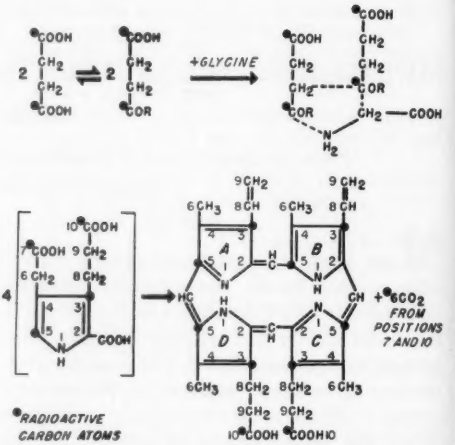


FIG. 4. Labeling pattern in protoporphyrin, showing positions of succinate and the α-carbon atom of glycine.

difficult for some time to understand the distribution of the α-carbon atom of glycine in the porphyrin molecule (four in the pyrrole rings and the four methene bridge carbon atoms, fig. 2). A possible explanation for the found distribution of the α-carbon atom of glycine was that the glycine was utilized for porphyrin synthesis via two pathways.

'x' → Pyrrole ring carbon atom

Glycine

'y' → Methene bridge carbon atom

Since the metabolic pattern of the α-carbon atom of glycine was similar to that of the 'C_1' compounds, and since the methene bridge carbon atoms are no longer attached to the nitrogen atom, it would appear that a 'C_1' compound might be substituted for compound 'y.' Studies were undertaken to test this point. In no case could CH_3OH, H_2CO, HCOOH, CH_3NH_2, CO_2 or CHO—COOH substitute for glycine. These negative findings, coupled with our finding that the α-carbon atom of glycine was always *equally* utilized for both the pyrrole-ring carbon atom and the methene-bridge carbon atom led us to conclude that the same derivative of glycine was utilized for both of these positions.

These findings limit the number of possible ways in which the succinate and glycine combine to form a pyrrole unit. The mechanism of con-

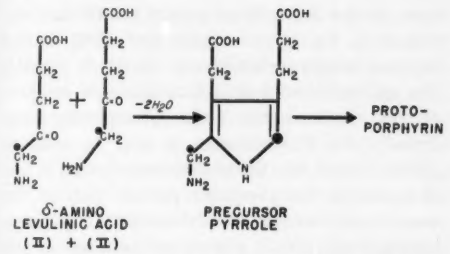


FIG. 5. Formation of the precursor pyrrole for porphyrins from two moles of δ-aminolevulinic acid.

densation of succinate and glycine becomes a bit more obvious if one considers that the product of this condensation should not only supply a reasonable mechanism of pyrrole formation, but should also suggest a reasonable mechanism by which the α-carbon atom of glycine is detached from its carboxyl group and should explain the distribution of the α-carbon atom of glycine in the porphyrin molecule. The condensation of succinate on the α-carbon atom of glycine to form α-amino-β-ketoadipic acid would appear to agree with the above findings. The compound formed, being a β-keto acid, could then readily be decarboxylated, and thus provide a mechanism by which the α-carbon atom of glycine becomes detached from its carboxyl group. It would also resolve the apparent paradox that while only the α-carbon atom of glycine is utilized for porphyrin synthesis, yet no 'C_1' compound can act as a substitute. The product of the decarboxylation would then be δ-aminolevulinic acid. Condensation of two moles of the latter, by a Knorr type of condensation (fig. 5), would readily give rise to a pyrrole in which the α-carbon atom of glycine would be distributed in the positions previously observed. To test this hypothesis α-aminolevulinic acid was synthesized in three independent ways (10). This was done to insure us of the correctness of the structure. The compound synthesized had the correct analysis and took up one mole of periodate. From the periodate reaction the expected products, formaldehyde and succinic acid, were isolated in excellent yields. Prior to our synthesis of δ-aminolevulinic acid, Wynn and Corwin (25) had prepared this compound by another series of reactions.

In the initial experiments, unlabeled δ-aminolevulinic acid was added to duck red blood cell hemolysates along with either C^{14}-labeled glycine or C^{14}-labeled succinate. The radioactivities of the hemin samples isolated in these experiments were compared with those obtained from controls in which the unlabeled δ-aminolevulinic acid was omitted. The rationale for these dilution-type of experiments is as follows: if δ-aminolevulinic acid is an intermediate formed from the condensation of glycine and succinate, any labeled δ-aminolevulinic acid formed from these labeled substrates will be diluted by the added unlabeled compound, and consequently this should be reflected in the lowered radioactivity of the hemin samples synthesized in the presence of unlabeled δ-aminolevulinic acid. It can be seen from table 1 that the hemin samples made in the presence of unlabeled δ-aminolevulinic acid contained less C^{14} than those of the controls made either from C^{14}-labeled glycine or succinate (10). These results, which are in full agreement with the hypothesis, can also be explained, however, by the possibility that δ-aminolevulinic acid is acting not as a diluent but as an inhibitor of heme synthesis. To rule out the latter possibility, the δ-aminolevulinic acid added in experiment 2 (table 1) was labeled with N^{15}. It can be seen

TABLE 1. COMPARISON OF C^{14} ACTIVITIES OF HEMIN SYNTHESIZED FROM GLYCINE-2-C^{14} AND SUCCINIC ACID-2-C^{14} IN PRESENCE AND ABSENCE OF NON-RADIOACTIVE δ-AMINOLEVULINIC ACID

Exp. No.	Labeled Substrate	Other Additions	Isotope Conc. in Hemin: C^{14} cpm	Isotope Conc. in Hemin: N^{15} atom per cent excess
1	C^{14}-Succinate (0.1 mM)		330	
	C^{14}-Succinate (0.1 mM)	δ-aminolevulinic acid 0.1 mM	90	
2	C^{14}-Glycine (0.05 mM)		230	
	C^{14}-Glycine (0.05 mM)	N^{15}-labeled δ-aminolevulinic acid 0.05 mM	48	0 21
	N^{15}-Glycine (0.33 mM)			0 05

TABLE 2. COMPARISON OF C^{14} ACTIVITIES IN HEMIN SYNTHESIZED FROM GLYCINE-2-C^{14} (0.05 MC/MM) AND δ-AMINOLEVULINIC ACID-5-C^{14} (0.05 MC/MM)

Labeled Substrate	Non-radioactive Additions	C^{14} Activity in Hemin cpm
C^{14}-Glycine (0.05 mM)	Succinate (0.1 mM)	480
C^{14}-δ-aminolevulinic acid (0.05 mM)		23,000
C^{14}-δ-amino-levulinic acid (0.05 mM)	Glycine (0.33 mM) Succinate (0.1 mM)	21,000

that whereas the incorporation of C^{14} from the glycine was lowered, there was a comparatively large incorporation of N^{15} into the porphyrin, thus demonstrating that the lowered C^{14} activity of the hemin sample was due to dilution rather than inhibition. Further proof that δ-aminolevulinic acid is a result of the condensation of glycine and succinate was obtained by incubating red blood cell hemolysates with glycine-2-C^{14} and unlabeled δ-aminolevulinic acid, and subsequently isolating the δ-carbon atom. In such an experiment it was found that the formaldehyde liberated upon periodate oxidation of a crude fraction containing δ-aminolevulinic acid was highly radioactive.

It now remained to establish more directly the utilization of δ-amino-levulinic acid for porphyrin formation. The compound was synthesized with C^{14} in its δ-carbon atom, and its utilization was compared with that of glycine. It can be seen from table 2 that the hemin synthesized from an equimolar amount of δ-aminolevulinic acid was about 65 times more radioactive than hemin synthesized from glycine. More rigorous proof that δ-aminolevulinic acid is indeed the precursor for porphyrin synthesis was obtained by degrading a hemin sample synthesized from δ-aminolevulinic acid-5-C^{14}. The δ-carbon atom of the latter compound should label the same carbon atoms of protoporphyrin as those which we have previously found to arise from the α-carbon atom of glycine, since according to the hypothesis the latter carbon atom is the biological source of the δ-carbon atom of δ-aminolevulinic acid.

It can be seen from table 3 that the same C^{14} distribution pattern was found in protoporphyrin synthesized from δ-aminolevulinic acid-5-C^{14} as from glycine-2-C^{14}; 50 per cent of the C^{14} activity resides in the pyrrole rings and 50% in the methene bridge carbon atoms (see fig. 2; ref. 11). The utilization of δ-aminolevulinic acid for porphyrin synthesis has been subsequently confirmed (26). Furthermore, it may be well to point out that the theoretical formulation of the structure of the precursor pyrrole (10) is the same structure which was determined for porphobilinogen (27–29), a compound excreted in the urine of patients with acute porphyria. The utilization of δ-aminolevulinic acid for porphyrin formation adds to the certainty that porphobilinogen (30) is an intermediate in porphyrin synthesis. These findings make α-amino-β-ketoadipic acid an obligatory intermediate.

TABLE 3. DISTRIBUTION OF C^{14} ACTIVITY IN PROTOPORPHYRIN SYNTHESIZED FROM δ-AMINOLEVULINIC ACID-5-C^{14} AND FROM GLYCINE-2-C^{14}

Fragments of porphyrin	Molar activity (%) in fragments of porphyrin synthesized from δ-Aminolevulinic acid-5-C^{14}, %	Glycine-2-C^{14}, %
Protoporphyrin	100	100
Pyrrole rings A + B (methylethylmaleimide)	24.5	24.6
Pyrrole rings C + D (Hematinic acid)	25.2	25.3
Pyrrole rings A + B + C + D	49.7	49.9
Methene bridge carbon atoms	50.3	50.1

The condensation of 'active' succinate and glycine to form δ-aminolevulinic acid subsequently thus far appears to require the partially intact structure of the red blood cell. It has been found that whereas δ-aminolevulinic acid can be converted to protoporphyrin in either an homogenized preparation or a cell-free extract, or in a lyophilized preparation of a cell-free extract (11), the conversion of succinate and glycine to porphyrin only takes place with intact cells or with those cells which have been hemolyzed with water. Homogenized preparations obtained in a blendor are no longer capable of synthesizing protoporphyrin from succinate and glycine (11). It would appear that on homogenization the functional activity of only those enzymes of the system that are involved in the condensation of succinate and glycine is lost.

If the proposed succinate-glycine cycle is a pathway of glycine metabolism embracing the reactions known for the α-carbon atom of glycine, then the δ-carbon atom of δ-aminolevulinic acid should have the same metabolic spectrum. To test the hypothesis, δ-aminolevulinic acid-5-C^{14} was injected into a duck, and hemin, guanine and adenine were isolated from the red blood cells, serine and the methyl group of methionine were obtained from the plasma proteins and uric acid was isolated from the excreta.

It was found that the ureido groups of the purines (Russell and Shemin, unpublished observations), the β-carbon atom of serine (Gatt and Shemin, unpublished observations), and the methyl group of methionine Foster and Shemin, unpublished observations contained C^{14}. These experimental results, which support the existence of the succinate-glycine cycle, are only to

be considered as preliminary in nature, since quantitative aspects have not yet been completely explored. Quantitative evaluation is difficult in whole animals, since cells are not very permeable to δ-aminolevulinic acid and the compound is very unstable as the free amino acid. In an experiment in the bird the C^{14} activity of both the hemin and the ureido groups of the purines synthesized from δ-aminolevulinic acid-5-C^{14} was 2.5 times greater than in those samples synthesized in a comparable experiment in which glycine-2-C^{14} was the substrate. This may be a minimal difference if one considers the experiments *in vitro* (see table 2) in which the C^{14} activity of the hemin synthesized from δ-aminolevulinic acid was about 65 times greater than that synthesized from glycine. However, it would appear from the above results that the δ-carbon atom of δ-aminolevulinic acid has the same metabolic pattern as that of the α-carbon atom of glycine and likewise as that of the 'C_1' compounds beyond the porphyrin synthesis. Further support for this conclusion is our finding that while both glycine-2-C^{14} and δ-aminolevulinic acid-5-C^{14} gave rise to radioactive formate in the urine of a rat, the formate arising from δ-aminolevulinic acid was about 3 times more radioactive than that made from the α-carbon atom of glycine (Gatt and Shemin, unpublished observations).

Although the evidence for the condensation of succinate and glycine appears to be rather conclusive, it does appear that this reaction may not be unique but rather a prototype of a more general reaction. We observed a few years ago that the utilization of glycine for porphyrin formation is markedly inhibited by the addition of either acetate or pyruvate (Labbe and Shemin, unpublished observations). These compounds appear to inhibit the condensation of succinate and glycine, since the conversion of δ-aminolevulinic acid to porphyrin is not influenced by the addition of these acids. It would appear that "active acetate may compete with succinate for the glycine, since the inhibition can be overcome by the addition of other members of the citric acid cycle. If acetate condenses with glycine the product would be α-aminoacetoacetic acid. Upon decarboxylation this β-keto acid would yield aminoacetone, an analogue of δ-aminolevulinic acid. We have carried out some studies with aminoacetone labeled with C^{14} in the carbon atom bearing the amino group. We have found that this radioactive carbon atom is utilized for the ureido groups of uric acid, that it can be converted both to formate and carbon dioxide the radioactivity of which is several times greater than that produced from glycine-2-C^{14}. It was found that aminoacetone can be synthesized in the animal (Gatt and Shemin, unpublished observations).

The condensation of glycine with either 'active' succinate or 'active' acetate provides a pathway whereby glycine can be oxidized to carbon dioxide and the intermediates produced in the cycle drawn off for the synthesis of other compounds. This is similar to the citric acid cycle, in which another two-carbon compound is oxidized to carbon dioxide and intermediates are produced which can be drawn off for synthesis. In the succinate-glycine cycle, succinate is the catalyst instead of oxaloacetate.

REFERENCES

1. Altman, K. I., G. W. Casarett, R. E. Masters, T. R. Noonan, and K. Salomon. *J. Biol. Chem.* 176: 319, 1948.
2. Radin, N. S., D. Rittenberg, and D. Shemin. *J. Biol. Chem.* 184: 745, 1950.
3. Wittenberg, J. and D. Shemin. *J. Biol. Chem.* 185: 103, 1950.
4. Muir, H. M. and A. Neuberger. *Biochem. J.* 47: 97, 1950.
5. Karlsson, J. L. and H. A. Barker. *J. Biol. Chem.* 177: 597, 1949.
6. Sakami, W. *J. Biol. Chem.* 178: 519, 1949.
7. Winnick, T., I. Moring-Claesson and D. M. Greenberg. *J. Biol. Chem.* 175: 127, 1948.
8. Arnstein, H. R. V. *Biochem. J.* 47: XVIII, 1950.
9. Weissbach, A., D. Elwyn and D. B. Sprinson. *J. Am. Chem. Soc.* 72: 3316, 1950.
10. Shemin, D. and C. S. Russell. *J. Am. Chem. Soc.* 75: 4873, 1953.
11. Shemin, D., T. Abramsky and C. S. Russell. *J. Am. Chem. Soc.* 76: 1204, 1954.
12. Shemin, D. and D. Rittenberg. *J. Biol. Chem.* 159: 567, 1945.
13. Shemin, D. and D. Rittenberg. *J. Biol. Chem.* 166: 621, 628, 1946.
14. Wittenberg, J. and D. Shemin. *Cold Spring Harbor Symposia Quant. Biol.* 13: 185, 1948.
15. Wittenberg, J. and D. Shemin. *J. Biol. Chem.* 178: 47, 1949.
16. Muir, H. M. and A. Neuberger. *Biochem. J.* 45: 163, 1949.
17. Grinstein, M., M. D. Kamen and C. V. Moore. *J. Biol. Chem.* 174: 767, 1948.
18. Shemin, D. and S. Kumin. *J. Biol. Chem.* 198: 827, 1952.
19. Shemin, D. and J. Wittenberg. *J. Biol. Chem.* 192: 315, 1951.

20. WRISTON, J. C., JR., L. LACK AND D. SHEMIN. *Federation Proc.* 12: 294, 1953.
21. SHEMIN, D., I. M. LONDON AND D. RITTENBERG. *J. Biol. Chem.* 173: 799, 1948.
22. SHEMIN, D., I. M. LONDON AND D. RITTENBERG. *J. Biol. Chem.* 183: 757, 1950.
23. SHEMIN, D. AND S. KUMIN. *Federation Proc.* 11: 285, 1952.
24. LONDON, I. M. AND M. YAMASAKI. *Federation Proc.* 11: 250, 1952.
25. WYNN, R. W. AND A. H. CORWIN. *J. Org. Chem.* 15: 203, 1950.
26. NEUBERGER, A. AND S. J. SCOTT. *Nature* 172: 1093, 1953.
27. WESTALL, R. G. *Nature* 170: 614, 1953.
28. COOKSON, G. H. AND C. RIMINGTON. *Nature* 171: 875, 1953.
29. KENNARD, O. *Nature* 171: 876, 1953.
30. FALK, J. E., E. I. B. DRESEL AND C. RIMINGTON. *Nature* 172: 292, 1953.

CHARACTERISTICS OF KWASHIORKOR[1] (SINDROME PLURICARENCIAL DE LA INFANCIA)

NEVIN S. SCRIMSHAW, MOISÉS BEHAR, GUILLERMO ARROYAVE, FERNANDO VITERI AND CARLOS TEJADA

From the Instituto de Nutrición de Centro América y Panamá (INCAP)[2]
Guatemala, Central America

SÍNDROME PLURICARENCIAL DE LA INFANCIA, as it is known throughout Latin America, or Kwashiorkor as it is called in most other areas of the world, is not only attracting increasing attention as a major disease entity but is also becoming much better understood. Indeed at the recent Princeton Conference on Protein Malnutrition in Children, sponsored by the Food and Agriculture Organization, the World Health Organization and the Josiah Macy Jr. Foundation, workers from seven different centers on three continents expressed a very large measure of agreement as to the characteristics of Kwashiorkor (1). After noting the clinical signs and symptoms and pathological findings generally agreed upon as characterizing the syndrome, we propose to review its known biochemical and physiological characteristics.

CLINICAL CHARACTERISTICS

Retarded growth and development are a consequence of the severe protein malnutrition characteristic of children with Kwashiorkor. It occasionally happens, however, that the process is so acute that the adverse effect on growth does not become manifest in the relatively short time before appearance of clinical symptoms. The changes of apathy and anorexia are also prominent clinical features and the rapid recovery from these symptoms is a good prognostic sign in treated cases. The child is also frequently hyperirritable. In all cases there is some degree of loss of muscle tone.

Edema is a constant finding and may often be very severe. The skin changes include atrophy, and areas of hyperkeratosis with pigmentation. Excoriations and secondary infection frequently may develop. The skin lesions are pellagroid in appearance but unlike those of pellagra are not confined principally to areas exposed to sunlight. Instead they are often severe over the trunk. Frequently acrocyanosis and a variegated pattern of erythema are observed in areas where the pellagroid lesions are not apparent.

The hair changes may include alterations of texture as well as of color. The hair is likely to be thin, dry, sparse and easily pulled out. It is usually abnormally pale and in some individuals acquires a reddish tint. The nails may be soft, irregular and show transverse lines of diminished growth.

PATHOLOGICAL CHARACTERISTICS

The liver shows fatty change which is often so severe as to involve almost every cell and obliterate the lumen of the sinusoids. A proliferation of the portal reticulum is also observed. Subsequently the proliferation increases producing a star-like pattern and, later on, a ring of reticulum fibers surrounds each lobule to produce a monolobular reticulosis. The pancreas may initially appear enlarged but is more generally found at autopsy to be atrophied. The acini always show moderate to severe atrophy and a reduction in the number of secretory granules. Fibrosis is likely to be present and may be severe.

Some degree of atrophy of the wall is found in the intestine and stomach and gross dilatation is frequent at autopsy. The skin and skin appendages show atrophy especially in the region of gross lesions. The skin is also characterized by hyper- and parakeratosis. Atrophy of the follicles of the thyroid and a decrease in colloid substance together with interstitial fibrosis appear to be particularly severe in Central American cases (2). This is probably related to the prevalence

[1] Part of this work was supported by grants from the Nutrition Foundation and the Williams-Waterman Fund of the Research Corporation. Support is now being given by research grant A-981 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service.

of endemic goiter in the same areas, but thyroid changes have also been described from Africa (3). The adrenal cortex shows atrophy and loss of lipids. Sometimes fatty degeneration of the kidney tubules is also encountered.

HEMATOLOGICAL FINDINGS

A mild to moderate degree of anemia is a constant finding in Kwashiorkor, but the type may be microcytic, normocytic or macrocytic. We believe that the basic anemia associated with the protein deficiency of this syndrome is normocytic or mildly macrocytic in type and associated with a hypoplasia of the erythroblastic series in the bone marrow. Such an anemia responds readily to the feeding of skim milk alone as determined by a marked reticulocyte response and the renewal of erythroblastic activity in the bone marrow, illustrated in figure 1.

The presence of hookworm or chronic malaria may result in the appearance of microcytic hypochromic anemia which requires supplementary iron for a satisfactory therapeutic response. Even when the anemia is normocytic or macrocytic it is probable that iron stores are also inadequate. At least we have often seen macrocytic anemias become microcytic as dietary treatment was continued without supplementary iron.

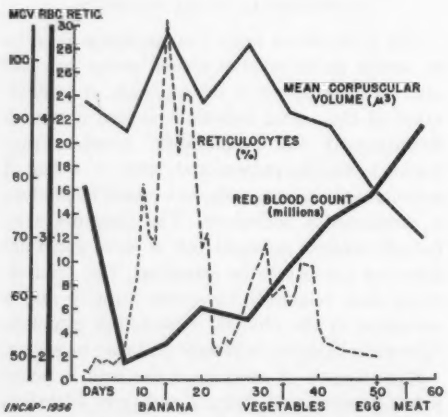


FIG. 1. Anemia in a 22-month-old girl with kwashiorkor. A prompt reticulocyte response was obtained from the administration of half skim milk alone with an accompanying increase in red blood cell count. The initial drop is due to hydration. When supplementary iron was given at the end of the period shown, a second reticulocyte response was obtained and the mean corpuscular volume returned to normal.

PARASITOLOGICAL FINDINGS

In general the intestinal parasites encountered are the same as those in children without Kwashiorkor in the same economic and social groups. Undoubtedly the parasitic infestation hastens the development of Kwashiorkor by interfering with the utilization of food, but there are no experimental studies to demonstrate this and the development of Kwashiorkor is not dependent on the presence of any particular species or combination of species of intestinal parasites.

BACTERIOLOGICAL FINDINGS

Studies in INCAP have identified Shigella organisms in 9.4% of 32 cases, but this is the same frequency with which they were recovered from non-Kwashiorkor cases hospitalized at the same time. However, since the diarrhea has usually been present for many weeks before hospitalization, the percentage of Shigella or Salmonella recoveries would be small even if the diarrhea were originally bacterial in origin.

Despite the relatively negative nature of the laboratory findings, there seems little doubt that infectious diarrhea plays an important part in the chain of events that leads a chronically malnourished child to develop frank Kwashiorkor. This has been discussed in detail in previous publications (4, 5).

BIOCHEMICAL FINDINGS

Total Protein in Blood. There is general agreement among all workers that serum total protein is low in Kwashiorkor and that if recovery is to occur the levels return to normal within two to three weeks after the initiation of good dietary treatment. The decrease is due principally to the lowering of serum albumin and hence the determination of albumin is a slightly more sensitive measurement of response to therapy. Since it has also been observed that the percentage of total globulin is increased, the albumin/globulin ratio is initially reversed and the normal ratio restored by therapy.

Electrophoretic studies (6–9) have demonstrated that the increase occurs in the alpha globulin fraction, particularly the alpha[1], as well as in the gamma globulin. The beta globulin component is little affected. Dean and Schwartz (6) have reported an unknown component mi-

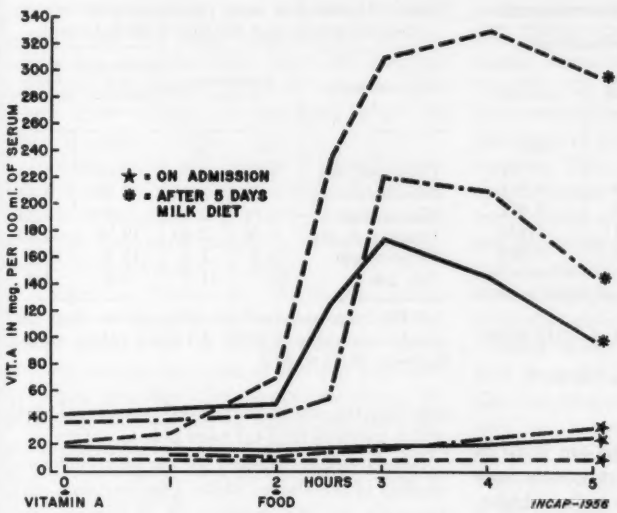


FIG. 2. Serum vitamin A in Kwashiorkor after oral administration of vitamin A palmitate. Administration of a single dose of 75,000 mcg. vitamin A palmitate by stomach tube produced no response in acute Kwashiorkor cases on admission, even when a test meal was given at the end of two hours. A marked increase in serum vitamin A was obtained in the same test following five days of treatment.

grating between the alpha[2] and beta fractions in several cases. In more detailed electrophoretic studies Close (7) reports that the curves suggest a number of additional protein fractions which may represent either normal components visible because of the low albumin values or the presence of unknown abnormal fractions.

Vitamins in Blood. *Vitamin A and carotene.* In Central America we have consistently found exceedingly low carotene and vitamin A serum levels (5, 8). The values determined by the method of Bessey *et al.* (10) are given in table 1 for nine recent cases. The values rise with good diet therapy to essentially normal limits in two to three weeks. Since vitamin A deficiency is common in Central America, but not in all areas of the world in which the syndrome occurs, it is not certain that low serum vitamin A and carotene levels will prove to be a constant finding. However, Moore and Sharman (11) and Trowell, Moore and Sharman in Uganda (12) also report very low carotene values in Kwashiorkor.

In view of the lowered duodenal enzyme activity and disturbed gastrointestinal function discussed subsequently, it is not surprising to find the absorption of fat soluble vitamins to be grossly abnormal. In our studies 75,000 micrograms of vitamin A palmitate in oil administered by stomach tube produced no detectable effect in five hours on the serum levels of children with Kwashiorkor, even when a meal was given two hours later as suggested by Mendeloff (13). As illustrated in the three cases in figure 2, a very marked and prompt response was observed to the same test following five days of treatment.

Vitamin E. The serum vitamin E levels are relatively low initially but also rise promptly and markedly with treatment as illustrated in table 1. (The method of Quaife *et al.* (14) was employed.) Trowell in Uganda (12) also reports low vitamin E values and believes that they reflect the generalized failure in intestinal absorption illustrated by the vitamin A and carotene findings.

Thiamine. No blood thiamine values have been previously reported for children with Kwashiorkor. The data presented in table 1 were obtained using the method of Burch *et al.* (15) and do not appear to be low. There is, however, a significant increase following three to five weeks of hospital treatment.

Riboflavin. Riboflavin values have been reported previously only from this laboratory (5, 8) and the average values stated to be within normal limits. Recent average values of assays

[2] A cooperative Institute for the study of human nutrition, supported by the Governments of Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama and administered by the Pan American Sanitary Bureau, Regional Office of the World Health Organization. INCAP Scientific Publication I-61.

TABLE 1. BLOOD VITAMIN LEVELS IN KWASHIORKOR

Constituent	No.	Content/100 ml	
		Upon admission	After recovery
		μg	
Carotene	9	5.0	139‡
Vitamin A	9	12.8	34.1‡
Vitamin E§	7	0.39	0.64‡
Thiamine*	4	5.23	6.83‡
Free riboflavin†	9	0.59	1.82‡
Total riboflavin	9	1.58	3.79‡

* Determined in whole blood—all other assays were made with blood serum.

† Free plus a very small amount of flavin mononucleotide.

‡ Difference is significant at the 1% level.

§ mg/100 ml.

for both total and 'free' fractions are given in table 1. The individual levels vary greatly from extremely low values to ones as high or higher than those found in normal individuals. This is true for both the 'free' or unhydrolyzed fraction (free riboflavin plus the flavin mononucleotide) and the total riboflavin (unhydrolyzed fraction plus the flavin adenine dinucleotide) as determined by the method of Burch *et al.* (16). The increase with treatment is significant, probably due to the high riboflavin content of the large amount of milk given.

Ascorbic acid. Although serum ascorbic acid values have been previously reported from this laboratory as lying within normal limits (5, 8), they are omitted from table 1 because the situation is proving to be more complex than at first realized. In many cases very high ascorbic acid values, as determined by the method of Lowry *et. al.*(17), employing the modification suggested by Goodland *et al.* (18), are obtained. It now appears probable that these high values in certain Kwashiorkor cases are an artifact due to some as yet unidentified substance. Whatever the cause, the apparent ascorbic acid values of those cases which are initially high drop markedly in the first few days of treatment before rising to the essentially normal levels found on discharge. No ascorbic acid values in Kwashiorkor have as yet been reported from other laboratories.

Vitamins in Tissues. We have determined vitamin A in the livers of nine cases of Kwashiorkor by an adaptation of the methods of Sobel and Werbin (19) and Ames *et al.* (20). The results, shown in table 2, are exceedingly low in comparison with the one excellently nourished

TABLE 2. LEVELS OF SOME LIVER CONSTITUENTS IN KWASHIORKOR (per 100 gm of fresh tissue)

Constituent	Kwashiorkor 9 cases		Moderately well nourished 1 case	Excellently nourished 1 case
	m	*s*		
Thiamine, mg	0.09	0.06	0.08	0.12
Riboflavin, mg	0.68	0.14	0.82	0.56
Niacin, mg	5.84	2.18	7.89	13.23
Vitamin A, mg	3.50	2.63	13.23	95.65
Protein, gm	10.9	1.4	15.2	18.7
Fat, gm	24.7	11.7*	2.6	4.4

* The large standard deviation is due to an unusually low value of 9.0%. All other values ranged between 18.6 and 37.1.

case and the one in fairly good nutritional state which we have thus far been able to obtain.

Niacin values obtained by the microbiological method of the United States Pharmacopeia (21), thiamine by the method of Hennessey and Cerecedo (22) and riboflavin by the method of Hodson and Norris (23) all closely resembled those in the child considered to be in fairly good nutritional state. They differed significantly from the excellently nourished case only in lower niacin values. Until more information is available on the range of values in normal children, caution is necessary for the interpretation of these data.

Minerals in Blood. The average values for blood serum calcium in 13 acute cases of Kwashiorkor recently studied by us was 10.1 mg/100 ml (s 2.0)[3]. For 11 of these cases the value for serum phosphorus was found to be 3.74 mg/100 ml (s 0.7). Both averages are similar to those found in the Belgian Congo (24). Although these values are considered to be within normal limits, slightly lower levels for calcium have been reported from Brazil (25) and Egypt (26).

Enzymes in Blood. *Alkaline phosphatase.* Average alkaline phosphatase values are low in Kwashiorkor cases on admission and rise rapidly with treatment (5, 6, 8). This is illustrated by the values in table 3, obtained by the method of Bessey *et al.* (27). Dean (28) has shown that in some cases there is actually a slightly further drop in alkaline phosphatase at the beginning of treatment, a decrease which is also seen in most Central American cases. He attributes

[3] These analyses were carried out in the Instituto Agropecuario Nacional, Guatemala, through the cooperation of Dr. Robert L. Squibb, Chief of the Department of Animal Husbandry and Nutrition.

this to two fractions, one activated by magnesium and the other inhibited by cyanide, which are relatively high while the principal alkaline phosphatase of blood serum is low. These drop rapidly on treatment while the latter is rising.

Amylase, lipase and esterase. Although measured in the blood, amylase, lipase and esterase have their principal origin in the pancreas, and therefore are useful indicators of altered pancreatic function in the syndrome.

Workers in Uganda (6), the Belgian Congo (29) and ourselves in Central America (8) find serum amylase low in Kwashiorkor and agree that its prompt rise is one of the best indicators of initial recovery. Representative values obtained by the method of Smith and Roe (30) are given in table 3. In our experience the amylase is very low in most fatal cases and fails to rise with treatment.

Serum lipase and esterase have been studied in India by Srinivasan and Patwardhan (31) and shown to be low in acute Kwashiorkor and to rise with treatment. Serum esterase has also been determined by Dean in Uganda (6) and the same results obtained.

Pseudo-choline esterase. The initial pseudocholine esterase values and their rise following treatment are also shown in table 3. A micro adaptation of the method of Reingold *et al.* was used (32). These findings are the same as those in Gambia (33), Jamaica (34, 35) and Uganda (6).

Duodenal Enzymes. Authors in Brazil (36), Hungary (37), Africa (38), India (39) and Mexico (40) have described the drastic decrease in the activity of duodenal enzymes in acute Kwashiorkor. Amylase, lipase and trypsin are all affected with values dropping nearly to zero and returning rapidly to normal in the first 10 days of treatment.

TABLE 3. BLOOD SERUM ENZYME LEVELS IN KWASHIORKOR

Enzyme	No.	Units/100 ml of serum	
		On admission	At discharge
Amylase*	15	41	114§
Alkaline phosphatase†	22	2.66	5.16§
Pseudo-cholinesterase‡	4	0.36	1.28§

* Smith and Roe Units (30).
† mM/1/hr. of *p*-nitrophenol (27).
‡ Mitchell Units (32).
§ Difference significant at the 1% level.

Enzymes in Tissues. Waterlow has studied the pseudo-choline esterase activity of liver biopsy tissue from malnourished children in Gambia (33). He found it to be low initially but to increase more than 100% when a milk diet was given to the child. In a subsequent paper he reported that cytochrome oxidase and lactic dehydrogenase were not affected but that transaminase in liver may rise (34). Sriramachari and Ramalingaswami (41) in India determined histochemically the alkaline phosphatase activity of liver biopsy tissue and found it to be increased in Kwashiorkor and to fall with treatment.

Other Biochemical Findings in Blood. *Cholesterol.* In both Uganda (6) and the Belgian Congo (29) the level of cholesterol in Kwashiorkor is very low, averaging approximately 88 mg/100 ml and increasing markedly with treatment. The ratio of free to total cholesterol fell rapidly in the first two weeks and remained more or less constant thereafter. Low cholesterol values in Kwashiorkor have also been reported from Brazil (25) and Morocco (42).

Lipids. 'Total' serum lipids were reported high in children with Kwashiorkor by Van Der Sar working in Curaçao (43). On the other hand the phospholipid levels reported by Carvalho in Brazil were low (36).

Urea. Dean and Schwartz in Uganda (6) report a low average blood serum urea of 16.2 mg/100 ml at the beginning of treatment succeeded by a rise to values which exceeded the normal level of 20 to 25 mg/100 ml

Other Biochemical Findings in Tissue. Preliminary findings for protein and fat in livers of children with Kwashiorkor are also given in table 2. The high fat contents are in agreement with those found in Gambia (44), Uganda (45), French West Africa (46) and India (47). Increased amounts of cholesterol in liver have also been reported from India (41, 47).

The French West African workers (46) report the methionine content of both liver and muscle to be normal. The low protein values cited in table 2 agree with reports from Jamaica (34) and French West Africa (46). In evaluating enzyme activity Waterlow has also determined that the ribonucleic acid in liver is low in relation to desoxyribonucleic acid and rises with treatment (28). This is evidence of an increase in cytoplasmic protein with treatment.

XUM

PHYSIOLOGICAL CHANGES

Nitrogen Balance. A recent study of 44 two-day nitrogen balance experiments on children suffering from severe Kwashiorkor in the Belgian Congo (48) found positive balances. About half of the nitrogen fed was retained when levels up to 3.6 gm of milk protein per kilogram were fed. Our own preliminary figures on cases after initial recovery are lower. Dean mentioned a child with Kwashiorkor who absorbed 72 and 79% respectively of nitrogen fed at the beginning and end of 25 days of treatment (49). Children who were malnourished but who did not necessarily have Kwashiorkor were studied in India (50, 51) and found to have a positive balance on a poor vegetarian diet containing rice despite a protein intake of only 1.2 gm/kg of body weight Positive balances in Kwashiorkor have also been found in West Africa (52) and Mexico (53). Bray (52) cites evidence to show that African children on a poor diet retain a higher portion of the total nitrogen it contains than do United States or European children.

Amino Acid Metabolism. We are indebted to the laboratory of Holt (54) for most of the limited information on amino acid metabolism in Kwashiorkor. These preliminary findings show the excretion of threonine in urine to be reduced and the ratios of isoleucine to leucine and of phenylalanine to tyrosine to be higher than normal. Since it has now been shown that a mixture of 18 amino acids will suffice to bring about initial recovery (55), it now appears established that a deficiency of amino acids or their imbalance is primarily responsible for the basic clinical syndrome.

Fat Balance. Fat balances were obtained in 45 children with Kwashiorkor studied in the Belgian Congo (48) on intakes ranging from 0.5 to 5.6 gm/kg A 'lower-than-normal-value' of 82% retention was obtained. Dean in Uganda (49) reports a child treated for 25 days on a soya-banana diet who increased his absorption of fat from 86 to 96%. Holt (53) reports one case studied in Mexico City with an absorption of 50% on admission and 80% following six weeks therapy. In a case treated for six weeks we found a fat retention of 71%. Another treated case which developed mild diarrhea during part of the five-day balance study retained only 60%.

Authors in South Africa (56), Uganda (38), Dakar(57), the Belgian Congo (24), Ceylon (58), India (28) and Curaçao (43) have mentioned the steatorrhea which is common in Kwashiorkor.

Blood Volume. Gómez and co-workers have studied 51 children with signs of Kwashiorkor in Mexico (59) by Evan's blue dye dilution method and conclude that the extracellular fluid (thiocyanate space) and plasma volume calculated in grams per kilogram of body weight are increased in malnutrition. However, when edema is present, the selection of the correct body weight to use in the formula for calculating blood volume and the correct weight to which blood volume should be related is always uncertain.

We encounter cases which despite their edema appear clinically to be dehydrated as determined by the condition of the mucous membranes, eyeballs and skin. This is supported by the initial drop in hematological values which is frequently observed and is illustrated in figure 1.

Electrolytes. From work in South Africa (60) and Uganda (61) it appears definite that Kwashiorkor is associated with a state of potassium depletion and usually low blood serum levels of potassium. Although this is probably due initially to the diarrhea, protein therapy in acute Kwashiorkor without supplementary potassium has been stated to result in potassium deficiency (61). Relatively low values for plasma chlorides have been reported from India (62) and the Belgian Congo (24).

Kidney Function. Gopalan (28) reports that only 5 out of 26 children with untreated nutritional edema in India had normal 24-hour urine outputs while the majority of the remainder had pronounced oliguria. This has also been our own clinical experience in cases of acute Kwashiorkor.

Cardiac Function. Abnormal electrocardiograph findings have been reported from Mexico (63), South Africa (64) and the Belgian Congo (24) to consist principally of low voltage and minor abnormalities.

Gopalan (28) reports from India not only a profound diminution in the amplitude of all deflexions but also occasional prolongation of the QT interval. Rarely, abnormalities of rhythm occur.

Endocrinological Findings. Very few studies of endocrinological alterations have been done. Gopalan attributes the oliguria of Kwashiorkor to a failure of the liver to destroy antidiuretic hormone (28) Certain features of the recovery syndrome described in Mexico by Gómez and co-workers (65) especially changing eosinophile count and hypertrichosis have been attributed to a rapid return to normal of pituitary function.

There have been several excellent studies of the endocrinological consequences of severe chronic malnutrition in adults which are pertinent to the subject but which cannot be reviewed here (66–68).

ETIOLOGY AND RELATION TO MARASMUS

The syndrome of Kwashiorkor in its classical form occurs in children whose caloric intake is not necessarily deficient, but whose protein intake is grossly inadequate in quality and quantity. However, many children in underdeveloped areas suffer from a very severe deficiency of both calories and protein, as well as all other nutrients, and develop the syndrome known as marasmus. Jelliffe (69) points out that the clinical picture of marasmus has been somewhat neglected because it lacks some of the dramatic attributes of Kwashiorkor. It should be clearly understood that many of the characteristics of Kwashiorkor, including a considerable proportion of the biochemical and physiological findings may also apply to marasmus. In both Mexico (observation by senior author in February 1956) and Jamaica (35) considerable attention has been given to this problem and it is now under intensive investigation by us. Not until thorough studies of marasmus comparable to those which have been described for Kwashiorkor have been completed, will it be possible to state what proportion of the findings are limited to one or the other of these conditions.

Even is this can be done, the problem is somewhat academic since any degree of combination or shading between marasmus and classical Kwashiorkor can and does occur. In fact the great majority of cases of Kwashiorkor in Latin America, India, the Belgian Congo and many other areas of the world represent just such a combination and are spoken of as the marasmic type of Kwashiorkor. Our knowledge of these conditions does not justify any attempt to discuss separately biochemical and physiological characteristics of the classical and marasmic types of Kwashiorkor in the present report. The most crucial clinical differences seem to lie in the marked degree of muscular wasting in the latter together with the loss of subcutaneous fat revealed when the edema has disappeared. The differences in dietary and social background of the two conditions have been well reviewed by Autret and Behar (4) and Trowell *et al.* (45).

It is not the function of this paper to discuss the etiology of Kwashiorkor, but it is clear that as our knowledge of the syndrome increases, more and more features of the condition which are local variants or inconstant findings are being related to specific factors apart from protein deficiency. Thus the primary characteristics of the relative deficiency of protein common to all cases and forms of Kwashiorkor are gradually becoming clearer. The recent work of Brock *et al.* in South Africa (55) showing that satisfactory initial recovery and clearing of the major clinical signs and symptoms can occur promptly with the administration of a mixture of pure amino acids, is a major contribution in this regard.

THE TASK AHEAD

The future work with Kwashiorkor would seem to lie in two mutually complementary directions: *1*) the development of practical prophylactic measures whereby young children can be given increased amounts of biologically adequate protein; *2*) the identification of specific etiological factors associated with the basic syndrome and with the various local or variable concomitant signs and symptoms.

The former will necessarily include the use of plant as well as animal protein resources and the latter will lead the investigator into the complex realm of the deficiency of specific amino acids and their inter-relationships with other amino acids, with vitamins and with the relative availability of calories.

SUMMARY

The clinical and pathological characteristics of the condition known as Síndrome Pluricarencial de la Infancia in Latin America and Kwashiorkor in most other parts of the world are briefly summarized and the presently available biochemical and physiological information regarding this syndrome is reviewed in detail.

Data obtained from studies in Central America pertaining to aspects of the condition which have not previously been described are given as follows: neither blood levels for thiamine nor serum levels for total and 'free' riboflavin are decreased. A very poor absorption of oral vitamin A was found in patients with Kwashiorkor on admission compared with normal absorption following five days of treatment. The results of assays for vitamin A, carotene, niacin, thiamine and riboflavin in the livers of seven fatal cases of Kwashiorkor are given. The excellent hematological response to protein therapy of the

normocytic or mildly macrocytic anemia most frequently encountered is also illustrated.

The continuous variation from classical Kwashiorkor to marasmus is stressed with the warning that many of the so-called characteristics of Kwashiorkor are also to be found in the latter.

REFERENCES

1. *Protein Requirements and Their Fulfillment in Practice.* Proceedings of a Conference in Princeton, N. J., 1955. Sponsored jointly by the Food and Agriculture Organization, World Health Organization and Josiah Macy Jr. Foundation, New York. In press.
2. Tejada Valenzuela, C. *Revista del Colegio Médico de Guatemala* 6: 1, 1955.
3. Davies, J. N. P. *Lancet* 254: 317, 1948.
4. Autret, M. and M. Behar. FAO Nutritional Studies No. 13, Food and Agriculture Organization of the United Nations, Rome, Italy, Oct. 1954.
5. Scrimshaw, N. S., M. Behar, C. Perez and F. Viteri. *Pediatrics* 16: 378, 1955.
6. Dean, R. F. A. and R. Schwartz. *Brit. J. Nutrition* 7: 131, 1953.
7. Close, J. *Ann. Soc. Belge Méd. Trop.* 33: 185, 1953.
8. Scrimshaw, N. S., M. Behar, M. Guzman, F. Viteri and G. Arroyave. *Federation Proc.* 14: 449, 1955.
9. Anderson, C. G. and A. Altmann. *Lancet* 260: 203, 1951.
10. Bessey, O. A., O. H. Lowry, M. J. Brock and J. A. Lopez. *J. Biol. Chem.* 166: 177, 1946.
11. Moore, T. and I. M. Sharman. *Brit. J. Nutrition* 5: 119, 1951.
12. Trowell, H. C., T. Moore and I. M. Sharman. *Ann. New York Acad. Sc.* 57: 734, 1954. (Cited from *Biol. Abst.* 29: 28286, 1955.)
13. Mendeloff, A. I. *J. Clin. Investigation* 33: 1015, 1954.
14. Quaife, M. L., N. S. Scrimshaw and O. H. Lowry. *J. Biol. Chem.* 180: 1229, 1949.
15. Burch, H. B., O. A. Bessey, R. H. Love and O. H. Lowry. *J. Biol. Chem.* 198: 477, 1952.
16. Burch, H. B., O. A. Bessey and O. H. Lowry. *J. Biol. Chem.* 175: 457, 1948.
17. Lowry, O. H., J. A. Lopez and O. A. Bessey. *J. Biol. Chem.* 160: 609, 1945.
18. Goodland, R. L., R. R. Sealock, N. S. Scrimshaw and L. C. Clark. *Science* 109: 494, 1949.
19. Sobel, A. E. and H. Werbin. *Indust. Eng. Chem. Anal. ed.* 18: 570, 1946.
20. Ames, S. R., H. A. Risley and P. L. Harris. *Anal. Chem.* 26: 1378, 1954.
21. *The Pharmacopeia of the United States of America*, 14th. rev., 1950.
22. Hennessey, D. J. and L. R. Cerecedo. *J. Am. Chem. Soc.* 61: 179, 1939.
23. Hodson, A. Z. and L. C. Norris. *J. Biol. Chem.* 131: 621, 1939.
24. Dricot, C., P. Beheyt and P. Charles. (*Ann. Soc. Belge Méd. Trop.* 31: 581, 1951. (Cited by Holemans, K. and A. Lambrechts, ref. 48.)
25. Carvalho, M., A. G. Pinto, M. M. Schmidt, N. Potsch and N. Costa. *J. Pediat., Rio de J.* 11: 395, 1945.
26. Hanafy, M. *J. Roy. Egyptian M. A.* 34: 470, 1951. (Cited by Trowell, Davies and Dean, ref. 45.)
27. Bessey, O. A., O. H. Lowry and M. J. Brock. *J. Biol. Chem.* 164: 321, 1946.
28. *Protein Malnutrition.* Proceedings of a Conference in Jamaica, 1953. Sponsored jointly by the Food and Agriculture Organization, World Health Organization and Josiah Macy, Jr. Foundation, New York. Cambridge: University Press, 1955.
29. Demaeyer, E. M. and H. Vanderborght. *Ann. Soc. Belge Méd. Trop.* 34: 417, 1954.
30. Smith, B. W. and J. H. Roe. *J. Biol. Chem.* 179: 53, 1949.
31. Srinivasan, P. R. and V. N. Patwardhan. *Lancet* 263: 864, 1952.
32. Reingold, J. G., L. G. Tourigny and V. L. Yonan. *Am. J. Clin. Path.* 23: 645, 1953. (Cited from *Chem. Abst.* 47: 10046, 1953.)
33. Waterlow, J. *Lancet* 258: 908, 1950.
34. Waterlow, J. C. In: *Liver Injury;* Transactions of the Eleventh Conference, New York 1953, p. 72.
35. Jelliffe, D. B., G. Bras and K. L. Stuart. *West Indian Med. J.* 3: 43, 1954.
36. Carvalho, M. *Hospital, Rio de Janeiro.* 32: 307, 1947.
37. Véghelyi, P. V. *Trans. 5th Congr. Ass. int. Pediat. Acta Pediat.* 36: 483, 1948.
38. Thompson, M. D. and H. C. Trowell. *Lancet* 262: 1031, 1952.
39. Achar, S. T. and V. Benjamin. *Antiseptic* Aug. 1951.
40. Gomez, F., R. R. Galvan, J. Cravioto, S. Frenk and J. Vazquez. *Bol. Méd. Hosp. Inf. Méx.* 10: 106, 1953. (Cited by Trowell, Davies and Dean, ref. 45.)
41. Sriramachari, S. and V. Ramalingaswami. *Ind. J. Pediat.* 20: 1, 1953.
42. Delon, J. *Maroc. Méd.* 30: 578, 1951. (Cited by Trowell, Davies and Dean, ref. 45.)
43. Van Der Sar, A. *Documenta Neerlandica et Indonésica de Morbis Tropicis* 3: 25, 1951.
44. Waterlow, J. C. Med. Res. Council, Special Report Series No. 263. London: His Majesty's Stationery Office, 1948.
45. Trowell, H. C., J. N. P. Davies and R. F. A. Dean. *Kwashiorkor.* (1st ed.). London: Edward Arnold Ltd., 1954.
46. Auffret, C. and F. Tanguy. *Bull. Méd. A.O.F.* 7: 43, 1950. (Cited by Trowell, Davies and Dean, ref. 45.)
47. Ramalingaswami, V., S. Sriramachari and P. G. Tulpule. *Lancet* 2: 661, 1952.
48. Holemans, K. and A. Lambrechts. *J. Nutrition* 56: 477, 1955.
49. Dean, R. F. A. *Bull. World Health Organ.* 9: 767, 1953.

50. Murthy, H. B. N., S. K. Reddy, M. Swaminathan and V. Subrahmanyan. *Brit. J. Nutrition* 9: 203, 1955.
51. Sur, G., S. K. Reddy, M. Swaminathan and V. Subrahmanyan. *Brit. J. Nutrition* 9: 210, 1955.
52. Bray, B. *Brit. J. Nutrition* 7: 3, 1953.
53. Holt, L. E., Jr. Data of Gomez, cited in personal communication to Princeton Conference (ref. 1).
54. Cheung, Mung W., D. I. Fowler, P. M. Norton, S. E. Snyderman and L. E. Holt, Jr. *J. Trop. Pediatrics* 1: 141, 1955.
55. Brock, J. F., J. D. L. Hansen, E. E. Howe, P. J. Pretorius, J. G. A. Davel and R. G. Hendrickse. *Lancet* 269: 355, 1955.
56. Gillman, J. and T. Gillman. *Perspectives in Human Malnutrition*. New York: Grune, 1951.
57. Bergouniou, J. L. and J. Trémolieres. *Bull. Soc. path. exot.* 45: 242, 1952.
58. Jayasekera, H. T. W., B. V. de Mel and H. Collumbine. *Ceylon J. Med. Sci.* 8: 1, 1951. (Cited by Trowell, Davies and Dean, Ref. 45.)
59. Gomez, F., J. Vasquez Santaella, R. Ramos Galvan, J. Cravioto and S. Frenk. *Am. J. Diseases Children* 87: 673, 1954.
60. Hansen, J. D. L. and J. F. Brock. *Lancet* 267: 477, 1954.
61. Thompson, M. D. *Lancet* 268: 1181, 1955.
62. Chauduri, K. G. *Trans. 5th. Congr. Ass. Int. Pediat. Acta Pediat.* 36: 110, 1948. (Cited by Trowell, Davies and Dean, ref. 45.)
63. De La Torre, J., S. Berber and L. Duran. *Bol. Méd. Hosp. Infant. Mex.* 6: 317, 1949. (Cited by Trowell, Davies and Dean, ref. 45.)
64. Jansen, E. and J. S. Le Roux. *S. Afr. J. Clin. Sci.* 1: 100, 1950.
65. Gomez, F., R. Ramos Galvan, J. Cravioto Muñoz. Hospital Infantil de México, n.d. (Trabajo de investigación realizado en el Hospital Infantil de México)
66. Zubiran, S. and F. Gómez-Mont. In: *Vitamins and Hormones. Advances in Research and Applications*, Vol. 11, edited by R. S. Harris, G. F. Marrian and K. V. Thiman. New York: Acad. Press, 1953, p. 97.
67. Gonzales Valero, E., J. Garcia Reyes and F. Gómez-Mont. *Revista de Invest. Clinica* 2: 179, 1950.
68. Keys, A., J. Brozek, A. Henschel, O. Mickelsen and H. Longstreet Taylor. *The Biology of Human Starvation*. Vol. II Minneapolis: Univ. of Minnesota Press, 1950.
69. Jelliffe, D. B. *Infant Nutrition in the Subtropics and Tropics*. Geneva: World Health Organization. Monograph Series No. 29, 1955.

NEW MEMBERS ELECTED, SPRING 1956

The American Physiological Society[1]

Edward L. Alpen, U. S. Naval Radiological Defense Lab., San Francisco, Calif.
Mario Altamirano, Columbia Univ., New York City.
Reubin Andres, Johns Hopkins Hosp., Baltimore, Md.
Morris H. Aprison, Galesburg State Research Hosp., Galesburg, Ill.
James W. Archdeacon, Univ. of Kentucky, Lexington.
W. D. Armstrong, Univ. of Minnesota, Minneapolis.
Robert C. Bahn, Natl. Insts. of Health, Bethesda, Md.
Edward L. Beckman, U. S. Navy, F.P.O., New York City.
George W. Brown, VA Hosp., Iowa City, Iowa.
Nancy L. R. Bucher, Harvard Univ., Boston, Mass.
Frank G. Carpenter, Cornell Univ., New York City.
Edward L. Chambers, Univ. of Miami, Coral Gables, Fla.
Margaret H. Clare, Washington Univ., St. Louis, Mo.
Leonard A. Cohen, Univ. of Pittsburgh, Pittsburgh, Pa.
Paris Constantinides, Univ. of British Columbia, Vancouver, Canada.
Homer E. Dale, Univ. of Missouri, Columbia.
B. Shannon Danes, Carlsberg Lab., Copenhagen, Denmark.
Vincent P. Dole, Rockefeller Inst., New York City.
Isidore S. Edelman, Univ. of California, San Francisco.
Earl Eldred, Univ. of California, Los Angeles.
Leonard H. Elwell, Univ. of Michigan, Ann Arbor.
Saul J. Farber, New York Univ., New York City.
Walter H. Freygang, Jr., Natl. Insts. of Health, Bethesda, Md.
Harry Goldberg, Hahnemann Med. College, Philadelphia, Pa.
Roger L. Greif, Cornell Univ., New York City.
Jacob Grossman, Montefiore Hosp., New York City.
Rodney B. Harvey, Univ. of Utah, Salt Lake City.
Edith D. Hendley, Univ. of Chicago, Chicago, Ill.
Attie Y. Hillier, Univ. of Chicago, Chicago, Ill.
Herbert Jonas, Univ. of Virginia, Charlottesville.
Frederick F. Kao, State Univ. of New York, New York City.
J. Gordon Kaplan, Dalhousie Univ., Halifax, Canada.
Lola S. Kelly, Univ. of California, Berkeley.
Donald J. Kimeldorf, U. S. Naval Radiological Defense Lab., San Francisco, Calif.
Howard M. Klitgaard, Marquette Univ., Milwaukee, Wis.
Howard P. Krieger, Mt. Sinai Hosp., New York City.
William M. Landau, Washington Univ., St. Louis, Mo.
Merle Lawrence, Univ. of Michigan, Ann Arbor.
Jerome Y. Lettvin, Massachusetts Inst. of Technology, Cambridge.
Warner E. Love, Inst. for Cancer Research, Philadelphia, Pa.
Aldo A. Luisada, Chicago Med. School, Chicago, Ill.
Miles D. McCarthy, Pomona College, Claremont, Calif.
Akira Omachi, Loyola Univ., Chicago, Ill.
Charles R. Park, Vanderbilt Univ., Nashville, Tenn.
Preston L. Perlman, Schering Corp., Bloomfield, N. J.
Henry L. Price, Univ. of Pennsylvania, Philadelphia.
Walter Redisch, New York Univ., New York City.
Kurt R. Reissmann, Univ. of Kansas, Kansas City.
Stelios C. Samaras, Creighton Univ., Omaha, Nebr.
Jurg A. Schneider, Ciba Pharmaceutical Prod., Inc., Summit, N. J.
Roger W. Sevy, Temple Univ., Philadelphia, Pa.
Oscar W. Shadle, Univ. of Louisville, Louisville, Ky.
Sol Sherry, Jewish Hosp. of St. Louis, St. Louis, Mo.
Theodore H. Spaet, Montefiore Hosp., New York City.
Joseph W. Still, George Washington Univ., Washington, D. C.
Marguerite N. Swift, U. S. Naval Radiological Defense Lab., San Francisco, Calif.

[1] Includes members elected Fall 1955.

Pei Chin Tang, Univ. of Texas, Dallas.
Ichiji Tasaki, Natl. Insts. of Health, Bethesda, Md.
Stephen M. Tenney, Univ. of Rochester, Rochester, N. Y.
David D. Thompson, Cornell Univ., New York City.
Louis Tobian, Univ. of Minnesota, Minneapolis.
Ramon Villareal, Univ. of San Luis Potosi, San Luis Potosi, Mexico.
George F. Warner, Univ. of California, San Francisco.
Seymour H. Wollman, Natl. Insts. of Health, Bethesda, Md.

American Society of Biological Chemists, Inc.

Samuel Abraham, Univ. of California, Berkeley.
Edward H. Ahrens, Jr., Rockefeller Inst., New York City.
Robert A. Alberty, Univ. of Wisconsin, Madison.
James Ashmore, Harvard Univ., Boston, Mass.
G. Gilbert Ashwell, Natl. Insts. of Health, Bethesda, Md.
Clinton E. Ballou, Univ. of California, Berkeley.
Harold W. Barrett, Univ. of Kansas, Lawrence.
Harry Beevers, Purdue Univ., Lafayette, Ind.
I. A. Bernstein, Univ. of Michigan, Ann Arbor.
Milan Bier, Fordham Univ., New York City.
Arthur L. Black, Univ. of California, Davis.
Jaques Bourdillon, New York State Dept. of Health, Albany.
Norman G. Brink, Merck and Co., Inc., Rahway, N. J.
Ray K. Brown, New York State Dept. of Health, Albany.
Helen B. Burch, Washington Univ., St. Louis, Mo.
John J. Burns, New York Univ., New York City.
E. S. Canellakis, Yale Univ., New Haven, Conn.
W. O. Caster, Univ. of Minnesota, Minneapolis.
Irwin Clark, Merck Inst., Rahway, N. J.
Cyril L. Comar, Oak Ridge Inst., Oak Ridge, Tenn.
Barry Commoner, Washington Univ., St. Louis, Mo.
George L. Curran, Univ. of Kansas, Kansas City.
George K. Davis, Univ. of Florida, Gainesville.
F. E. Deatherage, Ohio State Univ., Columbus.
Charles A. Dekker, Univ. of California, Berkeley.
E. A. Delwiche, Cornell Univ., Ithaca, N. Y.
Ralph D. DeMoss, Univ. of Illinois, Urbana.
Howard A. Eder, State Univ. of New York, Brooklyn.
Eric Ellenbogen, Univ. of Pittsburgh, Pittsburgh, Pa.
David Elson, Columbia Univ., New York City.
Bernard F. Erlanger, Columbia Univ., New York City.
Emmanuel Farber, Tulane Univ., New Orleans, La.
Earl Frieden, Florida State Univ., Tallahassee.
Wilhelm R. Frisell, Univ. of Colorado, Denver.
Charles E. Frohman, Lafayette Clinic, Detroit, Mich.
Oscar Gawron, Duquesne Univ., Pittsburgh, Pa.
David M. Gibson, Univ. of Wisconsin, Madison
Eugene Goldwasser, Univ. of Chicago, Chicago, Ill.
Bernard S. Gould, Massachusetts Inst. of Technology, Cambridge.
Harry Green, Wills Eye Hosp., Philadelphia, Pa.
Daniel L. Harris, Univ. of Chicago, Chicago, Ill.
Werner Hausmann, Rockefeller Inst., New York City.
Eugene L. Hess, Rheumatic Fever Research Inst., Chicago, Ill.
Mahlon B. Hoagland, Massachusetts General Hosp., Boston.
Robert W. Holley, Cornell Univ., Geneva, N. Y.
Miyoshi Ikawa, Univ. of California, Berkeley.
Maynard A. Joslyn, Univ. of California, Berkeley.
Arthur L. Koch, Univ. of Florida, Gainesville.
Bert N. La Du, Jr., Natl. Insts. of Health, Bethesda, Md.
Robert G. Langdon, Johns Hopkins Univ., Baltimore, Md.
Urban J. Lewis, Merck and Co., Inc., Rahway, N. J.
Arthur Lindenbaum, Argonne Natl. Lab., Lemont, Ill.
Alfred Linker, Columbia Univ., New York City.
Frank A. Loewus, U. S. Dept. of Agriculture, Albany, Calif.
Rufus Lumry, Univ. of Minnesota, Minneapolis.
Donald L. MacDonald, Univ. of California, Berkeley.
Guido V. Marinetti, Univ. of Rochester, Rochester, N. Y.
Vincent Massey, Henry Ford Hosp., Detroit, Mich.
Bert B. Migicovsky, Canada Dept. of Agriculture Ottawa.
Russell C. Mills, Univ. of Kansas, Lawrence.
L. A. Mounter, Med. College of Virginia, Richmond.
Walter L. Nelson, Cornell Univ., Ithaca, N. Y.
Peter Oesper, Hahnemann Med. College, Philadelphia, Pa.
Ernest L. Patterson, American Cyanamid Co., Pearl River, N. Y.
Edwin A. Popenoe, Brookhaven Natl. Lab., Upton, N. Y.
J. M. Price, Univ. of Wisconsin, Madison.
William H. Prusoff, Yale Univ., New Haven, Conn.

Richard E. Reeves, Louisiana State Univ., New Orleans.
Elizabeth Roboz, Georgetown Univ., Washington, D. C.
Robert S. Rosenfeld, Sloan-Kettering Inst., New York City.
Jay S. Roth, Hahnemann Med. College, Philadelphia, Pa.
Morton Rothstein, Univ. of California, Berkeley.
Richard S. Schweet, California Inst. of Technology, Pasadena.
F. Marott Sinex, Brookhaven Natl. Lab., Upton, N. Y.
Folke Skoog, Univ. of Wisconsin, Madison.
Lucile Smith, Univ. of Pennsylvania, Philadelphia.
Martin Sonenberg, Sloan-Kettering Inst., New York City.
Roger Y. Stanier, Univ. of California, Berkeley.
Alfred Saub, Univ. of Colorado Denver.
Daniel Steinberg, Natl. Insts. of Health, Bethesda, Md.
D. Ralph Strength, St. Louis Univ., St. Louis, Mo.
Cornelius F. Strittmatter, Harvard Univ., Boston, Mass.
Paul Talalay, Univ. of Chicago, Chicago, Ill.
Harris H. Tallan, Rockefeller Inst., New York City.
Alvin Taurog, Univ. of California, Berkeley.
Charles Terner, Worcester Fndn. for Exptl. Biology, Shrewsbury, Mass.
Frederick I. Tsuji, VA Hosp., Pittsburgh, Pa.
Bert L. Vallee, Peter Bent Brigham Hosp., Boston, Mass.
David F. Waugh, Massachusetts Inst. of Technology, Cambridge.
Eugene C. Weinbach, Natl. Insts. of Health, Bethesda, Md.
James R. Weisinger, Rockefeller Inst., New York City.
Sidney Weiss, Inst. for Cancer Research, Philadelphia, Pa.
Bernard Weissmann, Mt. Sinai Hosp., New York City.
Milton Winitz, Natl. Insts. of Health, Bethesda, Md.
Herbert H. Wotiz, Boston Univ., Boston, Mass.
Gearrd R. Wyatt, Yale Univ., New Haven, Conn.

American Society for Pharmacology and Experimental Therapeutics

Joseph F. Artusio, Jr., Cornell Univ., New York City.
David H. Baeder, Wyeth Inst., Philadelphia, Pa.
Lyle V. Beck, Univ. of Pittsburgh, Pittsburgh, Pa.
Oscar F. Davis, Loyola Univ., Chicago, Ill.
Anastasius S. Dontas, Univ. of Michigan, Ann Arbor.
William W. Douglas, Albert Einstein College of Medicine, New York City.
James G. Foulks, Univ. of British Columbia, Vancouver, Canada.
Jerome M. Glassman, Wyeth Inst., Philadelphia, Pa.
Abraham Goldin, Natl. Insts. of Health, Bethesda, Md.
Grace W. Gray, Marquette Univ., Milwaukee, Wis.
David Grob, Johns Hopkins Univ., Baltimore, Md.
Paul B. Hagen, Yale Univ., New Haven, Conn.
Max A. Heinrich, Jr., Univ. of South Dakota, Vermillion.
Justin B. Hoekstra, Bristol Labs., Inc., Syracuse, N. Y.
Leon Hurwitz, Food and Drug Admin., Washington, D. C.
Sumner M. Kalman, Stanford Univ., San Francisco,Calif.
Alexander Kandel, Tulane Univ., New Orleans, La.
David A. Karnofsky, Sloan-Kettering Inst., New York City.
Dorothy Karp, Cornell Univ., New York City.
Ruth R. Levine, Tufts Univ., Boston, Mass.
John P. Long, State Univ. of Iowa, Iowa City.
Samuel Mallov, State Univ. of New York, Syracuse.
Gilbert J. Mannering, Univ. of Wisconsin, Madison.
Tag E. Mansour, Louisiana State Univ., New Orleans.
Jack W. Miller, Univ. of Wisconsin, Madison.
Tom S. Miya, Univ. of Nebraska, Lincoln.
Neil C. Moran, Natl. Insts. of Health, Bethesda, Md.
Gilbert H. Mudge, Johns Hopkins Univ., Baltimore, Md.
Thomas B. O'Dell, Irwin, Neisler & Co., Decatur, Ill.
George T. Okita, Univ. of Chicago, Chicago, Ill.
Robert E. Parks, Jr., Univ. of Wisconsin, Madison.
Preston L. Perlman, Schering Corp., Bloomfield, N. J.
Donald F. Petersen, Univ. of Chicago, Chicago, Ill.
James B. Preston, State Univ. of New York, Syracuse.
David P. Rall, Natl. Insts. of Health, Bethesda, Md.

Jay Roberts, Cornell Univ., New York City.
Ira Rosenblum, Albany Med. College, Albany, N. Y.
Jack P. Saunders, Natl. Insts. of Health, Bethesda, Md.
Jean Scholler, Sloan-Kettering Inst., New York City.
Kenneth G. Scott, Univ. of California, San Francisco.
Parkhurst A. Shore, Natl. Insts. of Health, Bethesda, Md.
Jack L. Strominger, Washington Univ., St. Louis, Mo.
Edward E. Swanson, Eli Lilly and Co., Indianapolis, Ind.
Sidney Udenfriend, Natl. Insts. of Health, Bethesda, Md.
Chester W. White, Jr., Boston City Hosp., Boston, Mass.
Walther Wilbrandt, Univ. of Berne, Berne, Switzerland.
Wallace D. Winters, Marquette Univ., Milwaukee, Wis.

The American Society for Experimental Pathology

Charles H. Altshuler, St. Joseph's Hosp., Milwaukee, Wis.
Ellsworth C. Alvord, Jr., Baylor Univ., Houston, Texas.
Stephen B. Andrus, Harvard Univ., Boston, Mass.
James S. Arnold, Univ. of Utah, Salt Lake City.
Charles A. Ashley, Columbia Univ., New York City.
Sergio A. Bencosme, Queen's Univ., Kingston, Canada.
Kurt Benirschke, Harvard Univ., Boston, Mass.
Leslie R. Bennett, Univ. of California, Los Angeles.
James M. B. Bloodworth, Jr., Ohio State Univ., Columbus.
Jackson J. Clemmons, Western Reserve Univ., Cleveland, Ohio.
Jonathan Cohen, Children's Med. Ctr., Boston, Mass.
Jesus de la Huerga, Northwestern Univ., Chicago, Ill.
John T. Ellis, Cornell Univ., New York City.
Patrick J. Fitzgerald, State Univ. of New York, Brooklyn.
John H. Fodden, Univ. of South Dakota, Vermillion.
Frederick G. Germuth, Johns Hopkins Univ., Baltimore, Md.
James W. Goddard, Jefferson Med. College, Philadelphia, Pa.
Robert A. Good, Univ. of Minnesota, Minneapolis.
Robert T. Habermann, Natl. Insts. of Health, Bethesda, Md.
James A. Halsted, State Univ. of New York, Syracuse.
William J. Harrington, Washington Univ., St. Louis, Mo.
Charles Harris, Temple Univ., Philadelphia, Pa.
Gordon R. Hennigar, Med. College of Virginia, Richmond.
Arthur T. Hertig, Harvard Univ., Boston, Mass.
Samuel P. Hicks, Harvard Univ., Boston, Mass.
Willard T. Hill, Northwestern Univ., Chicago, Ill.
J. Raymond Hinshaw, Univ. of Rochester, Rochester, N. Y.
Robert B. Jennings, Northwestern Univ., Chicago, Ill.
E. Elizabeth Jones, Wellesley College, Wellesley, Mass.
Henry S. Kaplan, Stanford Univ., San Francisco, Calif.
Roy Korson, Univ. of Vermont, Burlington.
Marvin Kuschner, New York Univ., New York City.
Edwin M. Lerner, II, Natl. Insts. of Health, Bethesda, Md.
Gerald A. LoGrippo, Wayne Univ., Detroit, Mich.
Louise S. Lombard, Natl. Insts. of Health, Bethesda, Md.
Sarah A. Luse, Washington Univ., St. Louis, Mo.
Guido Majno, Harvard Univ., Boston, Mass.
Earl J. Mason, Western Reserve Univ., Cleveland, Ohio.
Donald G. McKay, Boston Lying-In Hosp., Boston, Mass.
John B. Miale, Univ. of Miami, Coral Gables, Fla.
Edwin T. Nishimura, Northwestern Univ., Chicago, Ill.
Robert M. O'Neal, Washington Univ., St. Louis, Mo.
Theodore C. Panos, Univ. of Texas, Galveston.
Richmond T. Prehn, Natl. Insts. of Health, Bethesda, Md.
Donald A. Rowley, Univ. of Chicago, Chicago, Ill.
Agnes B. Russfield, Harvard Univ., Boston, Mass.
F. M. Schabel, Jr., Med. College of Alabama, Birmingham.
John A. Schilling, Univ. of Rochester, Rochester, N. Y.
John L. Shapiro, Vanderbilt Univ., Nashville, Tenn.
Philippe Shubik, Chicago Med. School, Chicago, Ill.
Ira Singer, Rockefeller Inst., New York City.
Leon Sokoloff, Natl. Insts. of Health, Bethesda, Md.

Benjamin H. Spargo, Univ. of Chicago, Chicago, Ill.
Frederic W. Stamler, State Univ. of Iowa, Iowa City.
Mario Stefanini, Tufts Univ., Boston, Mass.
Frederick Stohlman, Jr., Natl. Insts. of Health, Bethesda, Md.
Edgar B. Taft, Harvard Univ., Boston, Mass.
Alan P. Thal, Univ. of Minnesota, Minneapolis.
Wilbur A. Thomas, Washington Univ., St. Louis, Mo.
Helene W. Tollan, Sloan-Kettering Inst., New York City.
Gordon F. Vawter, School of Aviation Medicine, USAF, Randolph Field, Texas.
Bernard M. Wagner, Children's Hosp. of Philadelphia, Philadelphia, Pa.
Roy L. Walford, Jr., Univ. of California, Los Angeles.
Alvar A. Werder, Univ. of Kansas, Kansas City.
Jean K. Weston, Parke, Davis & Co., Detroit, Mich.
William L. Williams, Univ. of Tennessee, Memphis.

American Institute of Nutrition

Lucien A. Bavetta, Univ. of Southern Californiaz Los Angeles.
D. E. Becker, Univ. of Illinois, Urbana.
Louise J. Daniel, Cornell Univ., Ithaca, N. Y.
Burt W. Heywang, USDA Southwest Poultry Exper. Sta., Glendale, Ariz.
Edward G. High, Meharry Med. College, Nashville, Tenn.
Herman F. Kraybill, Fitzsimons Army Hosp., Denver, Colo.
Robert J. Lillie, USDA Animal and Poultry Husbandry Research Branch, Beltsville, Md.
Olaf Mickelsen, Natl. Insts. of Health, Bethesda, Md.
O. Neal Miller, Tulane Univ., New Orleans, La.
Lura M. Odland, USDA State Exper. Stations Div., Washington, D. C.
H. M. Scott, Univ. of Illinois, Urbana.
Martha F. Trulson, Harvard Univ., Boston, Mass.
Walter G. Unglaub, Tulane Univ., New Orleans, La.
Theodore B. Van Itallie, Harvard Univ., Boston, Mass.
Robert Van Reen, Johns Hopkins Univ., Baltimore, Md.
Charlotte M. Young, Cornell Univ., Ithaca, N. Y.

American Association of Immunologists

A. E. Axelrod, Univ. of Pittsburgh, Pittsburgh, Pa.
Abram S. Benenson, Walter Reed Army Med. Ctr., Washington, D. C.
Edward H. Bloch, Western Reserve Univ., Cleveland, Ohio.
Milton H. Brown, Univ. of Toronto, Toronto, Canada.
David M. Donaldson, Brigham Young Univ., Provo, Utah.
Solon A. Ellison, Columbia Univ., New York City.
Henry S. Fuller, Walter Reed Army Med. Ctr., Washington, D. C.
Justine S. Garvey, California Inst. of Technology, Pasadena.
Horace M. Gezon, Univ. of Pittsburgh, Pittsburgh, Pa.
Melvin H. Kaplan, Harvard Univ., Boston, Mass.
Sidney Leskowitz, Harvard Univ., Boston, Mass.
Lawrence Levine, New York State Dept. of Health, Albany.
Chen Pien Li, Natl. Insts. of Health, Bethesda, Md.
Robert B. Lindberg, Walter Reed Army Med. Ctr., Washington, D. C.
Clayton G. Loosli, Univ. of Chicago, Chicago, Ill.
Takashi Makinodan, Oak Ridge Natl. Lab., Oak Ridge, Tenn.
Alice E. Moore, Cornell Univ., New York City.
Councilman Morgan, Columbia Univ., New York City.
Louis H. Muschel, U. S. Army, A.P.O. 343, San Francisco, Calif.
Otto J. Plescia, Rutgers Univ., New Brunswick, N. J.
Noel R. Rose, Univ. of Buffalo, Buffalo, N. Y.
R. Walter Schlesinger, St. Louis Univ., St. Louis, Mo.
John H. Schwab, Univ. of North Carolina, Chapel Hill.
Georg F. Springer, Univ. of Pennsylvania, Philadelphia.
Mario Stefanini, Tufts Univ., Boston, Mass.
William D. Tigertt, Walter Reed Army Med. Ctr., Washington, D. C.

CORRECTIONS AND ADDITIONS TO MARCH ISSUE

Page 6. AZIMA AND GRAD, *1st line* should read: "1½–2 gm/kg" instead of "4 mg/kg".

Page 15. BERGLUND *et al.* The following revised abstract should be substituted for that printed.

Effect of vagal stimulation and acetylcholine on contractility of ventricles and on coronary blood flow in the dog. ERIK BERGLUND, GUNTHER SCHREINER, FRANK DUFF AND HANS BORST (introduced by WILLIAM H. FORBES). *Dept. of Physiology, Harvard School of Public Health, Boston, Mass.*

In dogs anesthetized with morphine and chloralose-urethane, the chest was opened and flowmeters inserted for measurement of aortic and left coronary artery blood flow. Pressures were measured in right and left auricles, pulmonary artery and aorta. After section of both vagi in the neck, the right and/or left vagus nerve (distal end) were stimulated electrically, producing a fall in heart rate from 145–210/min. to 100/min. or lower. When the heart rate was maintained at a constant high level by simultaneous electrical stimulation of one auricle, vagal stimulation was accompanied by a rise in atrial pressure and fall in arterial pressure and output. Coronary artery flow fell only when arterial pressure fell. Marked inhibition of atrial contraction was noted by direct vision. Infusion of acetylcholine 22–36 γ/kg/minute into the vena cava was accompanied by marked to moderate systemic vasodilation and lowering of the ventricular function curves, i.e. less stroke work was obtained at a given filling pressure. Heart rate was unchanged or decreased slightly. Coronary flow per minute work and per aortic pressure was not increased; this indicates that acetylcholine did not produce coronary vasodilation.

Page 20. BLUM AND COOK. *Last line* should read "increases" instead of "reduces."

Page 21. BOND AND EASTERDAY (introduced by CRONKITE). *Seventh and eighth lines:* delete "sec".

Page 45. DEAL, CHAKALES AND GREEN. Title should read: Adrenergic activity of canine hepatic vascular bed.

Pages 88 and 450. HART, LANGFITT AND MARRAZZI AND LANGFITT, HART AND MARRAZZI. Certain references to adrenochrome are in error. Adrenochrome does not prevent the inhibitory action of serotonin and all indoles employed thus far exert the same effect as adrenochrome on the transcallosal evoked potential though quantitatively they differ.

Page 126. MASUDA (introduced by Wheelon). *Address* should read: "*Dept. of Psychiatry and Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.*"

Page 171. SILBERBERG AND CATCHPOLE. *Line 7 from end should read:* "1.0%" instead of "0.2%".

Page 176. SPIEGEL, SZEKELY AND BAKER. *Second column, line 9* should read: "100 m sec" instead of "100 μ sec.

Page 249. FAIRHURST, SMITH AND GAL. *Fifth line from end of abstract* should read: "pseudocholinesterase of pigeon brain, but produced slight inhibition of the cholinesterase."

Page 250. FIELD *et al. Fourth line from end* should read: "Actinomycin C obtained from *Streptomyces Chrysomallus*" instead of "Actinomycin C obtained from *Aspergillus fumigatus.*"

Page 261. GHOSH (introduced by DAVIS). *Names of authors* should read: J. J. GHOSH, E. ADAMS AND B. D. DAVIS.

Page 307. H. R. MAHLER AND JOYCE DOUGLAS. *Tenth line from end* should read: "equal, there is an effect of the hydrogen para to the . . ."

Page 580. ADLER. This paper was presented on the program entitled **Antigens and Antibodies I,** on Tuesday, April 17.

Page 590. GOODGAL AND HERRIOTT. The following revised abstract should be substituted for that printed:

Uptake of transforming DNA and its penetration into Hemophilus influenzae. SOL H. GOODGAL AND ROGER M. HERRIOTT (introduced by B. F. CHOW). *Dept. of Biochemistry, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.*

The uptake of P^{32}-labelled transforming DNA by susceptible *Hemophilus influenzae* follows two different patterns. Some of the adsorbed DNA and TP is resistant to DNAse or washing and the quantity is directly related to the number of cells transformed. The remainder of the DNA and TP can be washed off or digested by DNAse and the quantity does not appear to be related to the number of cells transformed. In the latter case the eluted DNA and TP are equivalent to a fraction of the original TP solution, i.e., there was no fractionation or destruction by the uptake and

elution. Shortly after adsorption, but before phenotypic expression has taken place, biologically active TP can be obtained from disrupted cells; the TP is now DNAse sensitive and TCA precipitable. Assuming a molecular weight for DNA of 6,000,000 the following relationships may be noted. (1) A single cell of *Hemophilus influenzae* contains approximately 300 molecules of DNA. (2) The uptake of DNAse resistant DNA by the cell population involves 350 molecules per transformation. (3) The maximum frequency of transformation in our hands is one per 350 cells. The significance of these and other findings will be discussed. (This work was supplemented by a grant from the Atomic Energy Commission.)

Page 616. TABACHNICK AND CERCEO. *Title* should read: "Direct determination of ribonuclease and deoxyribonuclease activity in guinea-pig epidermal homogenates."

Pages iv and 768, author index. *The following reference* was omitted: Cornatzer, W. E., 767, 1775.

Pages vii and 771, author index. *The following references:* 1223, 1920; 1799, 1814 should be listed after Goldsmith, G. A., not Goldsmith, E. D.

Page 654. The following papers were added to the program on **Physiology in Undergraduate Colleges—Session on Teaching**—of the Physiology Society: Is an undergraduate major in physiology worthwhile? RUTH E. CONKLIN (*Vassar College*); and Devices for laboratory teaching of physiology, H. E. EDERSTROM AND B. DEBOER (*North Dakota Med. School*).

2 NEW WATERS INSTRUMENTS

RG-6 INSTANT SELF-DEVELOPING PHOTOKYMOGRAPH

...A new type recorder combining extreme sensitivity of photographic method with immediate visual record of direct writing method.

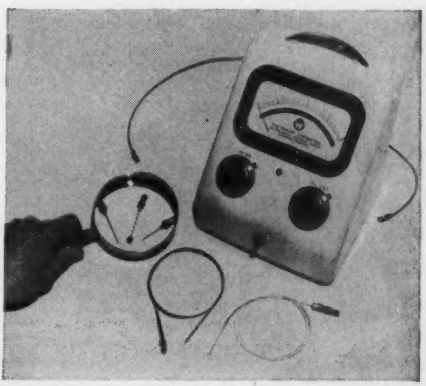
...Eliminates need of costly amplifier when recording small outputs.

Galvanometer light beam traces are developed on special 6 inch wide sensitized paper inside camera itself within a few seconds. Number of channels limited only by user's ability to read the multiple records. 3 speeds: 1 mm/sec, 5 mm/sec, and 25 mm/sec. Includes necessary light sources, mirrors for 1 or 2 meter light beam lengths plus 1 and 10 sec. time indicator.

TT-11 CONRAD THERMISTOR

...An extremely fast responding, versatile, resistance thermometer featuring unique probes for precision temperature measurement in all ranges.

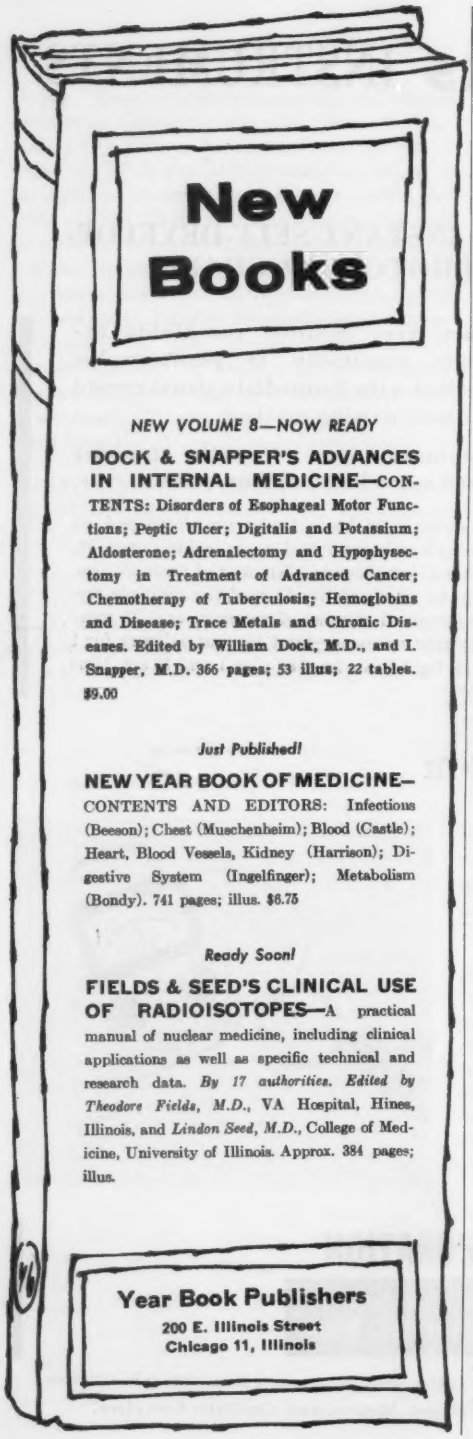
Exclusive 25 ga. and 22 ga. needle probes are unequaled in sensitivity. Also high quality, reliable polyethylene multi-purpose probe and skin surface probe. All probes sterilizable. Several can be used together for rapid consecutive readings through the one control box. Temperature range can be expanded or contracted, raised or lowered by the user. Provision for simultaneous recording.



THE WATERS CORPORATION

Electro-Medical Instrument Division
402 First Ave. N.W., Rochester, Minn.

Specialists in instrumentation for cardiovascular and pulmonary function laboratories. Other products include Oximeters, Nitrogen Meters, and Cardiotachometers.



NEW VOLUME 8—NOW READY

DOCK & SNAPPER'S ADVANCES IN INTERNAL MEDICINE—CONTENTS: Disorders of Esophageal Motor Functions; Peptic Ulcer; Digitalis and Potassium; Aldosterone; Adrenalectomy and Hypophysectomy in Treatment of Advanced Cancer; Chemotherapy of Tuberculosis; Hemoglobins and Disease; Trace Metals and Chronic Diseases. Edited by William Dock, M.D., and I. Snapper, M.D. 366 pages; 53 illus; 22 tables. $9.00

Just Published!

NEW YEAR BOOK OF MEDICINE—CONTENTS AND EDITORS: Infectious (Beeson); Chest (Muschenheim); Blood (Castle); Heart, Blood Vessels, Kidney (Harrison); Digestive System (Ingelfinger); Metabolism (Bondy). 741 pages; illus. $6.75

Ready Soon!

FIELDS & SEED'S CLINICAL USE OF RADIOISOTOPES—A practical manual of nuclear medicine, including clinical applications as well as specific technical and research data. *By 17 authorities. Edited by Theodore Fields, M.D.*, VA Hospital, Hines, Illinois, and *Lindon Seed, M.D.*, College of Medicine, University of Illinois. Approx. 384 pages; illus.

Year Book Publishers
200 E. Illinois Street
Chicago 11, Illinois

FOURTEENTH ANNUAL MEETING

The American Psychosomatic Society will hold its Fourteenth Annual Meeting at Chalfonte-Haddon Hall in Atlantic City on Saturday and Sunday, May 4 and 5, 1957.

The Program Committee would like to receive titles and abstracts of papers for consideration for the program no later than December 1, 1956. The time allotted for presentation of each paper will be twenty minutes.

Abstracts, in sextuplicate, should be submitted for the Program Committee's consideration to the Chairman, at 551 Madison Avenue, New York 22, New York.

I. Arthur Mirsky, M.D.
Chairman, Program Committee
Henry M. Fox, M.D.
Theodore Lidz, M.D.
Morton F. Reiser, M.D.
Eric D. Wittkower, M.D.

Radioactive Compounds

Limited supplies of radioactive cortisone, hydrocortisone and corticosterone (Compound B) are now available without charge to qualified investigators. All are labeled at position 4 with C^{14}. The cortisone has a specific activity of 0.49 mc/μM and the hydrocortisone and corticosterone a specific activity of 1.47 mc/μM. In addition, there are also available five intermediates in the synthesis of cortisone.

Requests, in the form of a letter concisely describing the proposed research, should be sent to

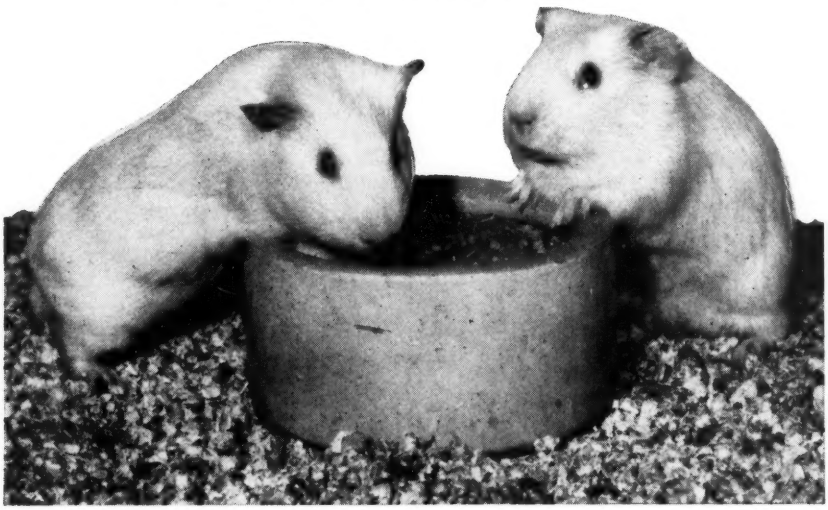
Endocrinology Study Section
Division of Research Grants
National Institutes of Health
Bethesda 14, Maryland

Transfer and use of these materials are subject to current Atomic Energy Commission regulations.

XUM

Purina *laboratory animal* Nutrition News

NOW, A COMPLETE CHOW FOR GUINEA PIGS



Purina Research has just released a new complete feed for guinea pigs, requiring no ascorbic acid or vegetables as a supplement. Purina Guinea Pig Chow contains all the nutrients guinea pigs need for good reproduction, lactation, growth and health. It is made in 5/32", Bite-Size Checker form.

This is an important step forward in the feeding of laboratory guinea pigs. It has always been a nuisance to provide the essential Vitamin C or ascorbic acid requirements of guinea pigs by feeding vegetables containing this vitamin or by feeding it through the drinking water.

The Vitamin C used in Purina's new Guinea Pig Chow has been stabilized to hold its potency more than 30 days after it reaches your laboratory. In ordering Guinea Pig Chow, you can estimate your needs by figuring the amount needed per animal at 1½ to 3 lbs. per month, depending on age.

This new, easy-to-feed, complete Guinea Pig Chow can be ordered through any one of the more than 5,000 Purina Dealers, located from coast to coast.

For further information about feeding laboratory guinea pigs and for the analysis of Guinea Pig Chow, write to Mr. Albert H. Leonard, Special Industry Sales, Ralston Purina Company, St. Louis 2, Mo.

RALSTON PURINA COMPANY, St. Louis 2, Missouri

With Over 5,000 Dealers Coast to Coast To Serve You

XUM

Packard Instruments

1 Liquid Scintillation Spectrometers

2 Automatic Fraction Collectors

3 Windowless and Flo-Window Counters

1 TRI-CARB LIQUID SCINTILLATION SPECTROMETERS

For counting Tritium, Carbon-14 and other beta emitting isotopes.

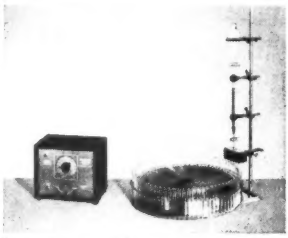
The Tri-Carb Spectrometer is simple to use. Most samples are easily prepared and hundreds per day can be readily handled. In addition to samples that are soluble in the scintillation solvent, it is also possible to count many entirely different classes of materials. Aqueous samples, completely insoluble solids, CO_2, H_2S, SO_2, and heavy metals can all be counted efficiently by recently developed sample preparation techniques.

Request Bulletin 314

2 AUTOMATIC FRACTION COLLECTORS

For precise column chromatography.

Provides both time and drop counting. Can be furnished for time operation only at commensurately lower cost.

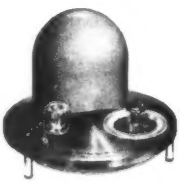


Drops from column fall directly into test tubes. There are no intermediate collecting vessels, glass arms, or funnels to cause mixing, contamination, evaporation, etc. This is important where accurate separations are required or where radioactive tracers are used.

Request Bulletin 230

3 WINDOWLESS AND FLO-WINDOW COUNTERS

Both types can be used for Geiger and proportional operation.



Windowless Flow Counter, Model 200A, provides maximum sensitivity for counting solid samples which emit very soft radiations. Has essentially unlimited life. Physical arrangement of sample in chamber makes it possible to achieve full 2π geometry.

Flo-Window Counter, Model 210, features a very thin metalized window of Du Pont Mylar which offers a minimum of obstruction to low energy radiation. Isolates counting chamber from sample. Eliminates static charge, vapor effects, accidental contamination, etc.

Request Bulletin 200

Packard Instrument Company

DEPT. C • P. O. BOX 428 • LA GRANGE, ILLINOIS